

FERMENTATION OF DIETARY FIBRE IN THE INTESTINAL TRACT

Margareta Nyman



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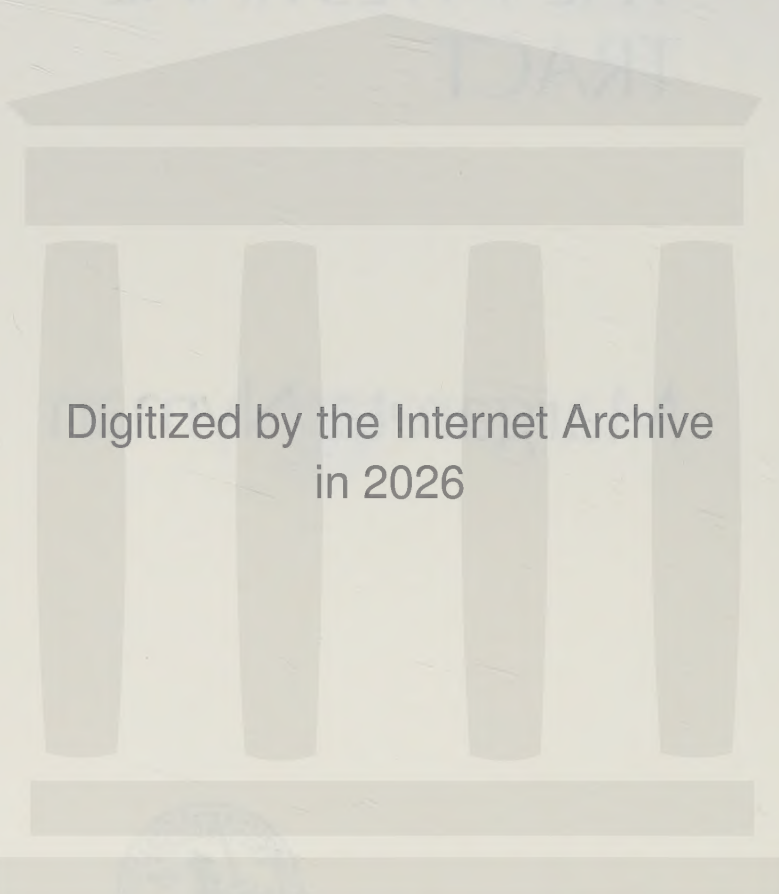


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This dissertation is based on the following papers, referred to by Roman numerals in the text.

- I Fermentation of Dietary Fibre in the Rat Intestinal Tract.
Nyman M & Asp N-G.
Br J Nutr 1982, 47, 357-366.
- II Dietary Fibre Fermentation in the Rat Intestinal Tract. Effect of Adaptation Period, Protein and Fibre levels, and Particle size.
Nyman M & Asp N-G.
Br J Nutr 1985, in press.
- III Fermentation of Dietary Fibre in the Intestinal Tract. - Comparison between Man and Rat.
Nyman M, Asp N-G, Cummings JH & Wiggins H.
Submitted for publication.
- IV Dietary Fiber Content and Composition in Six Cereals at Different Extraction Rates.
Nyman M, Siljeström M, Pedersen B, Bach Knudsen KE, Asp N-G, Johansson CG & Eggum BO.
Cereal Chem 1984, 61, 14-19.
- V Fermentation of Dietary Fibre in the Intestinal Tract of Rats. - A Comparison of Flours with Different Extraction Rates from Six Cereals.
Nyman M, Asp N-G, Pedersen B & Eggum BO.
J Cereal Sci 1985, 3, in press.
- VI Extrusion Cooking and Dietary Fiber: Effect on Dietary Fiber Content and on Degradation in the Rat Intestinal Tract.
Björck I, Nyman M & Asp N-G.
Cereal Chem 1984, 61, 174-179.
- VII Bulk Laxatives. - Their Dietary Fibre Composition, Degradation and Faecal Bulking Capacity in the Rat.
Nyman M & Asp N-G.
Scand J Gastroent 1985, in press.

INTRODUCTION

In the gastro-intestinal tract of man, protein, starch and fat are hydrolysed by the digestive enzymes and absorbed. After consumption of plant material a residue, consisting mainly of non-starch polysaccharides and lignin, passes unchanged into the colon. This residue is referred to as dietary fibre. Originally dietary fibre was considered to be an inert material without physiological importance. In the early 1970's, however, interest in dietary fibre increased. This can, to a large extent, be attributed to Burkitt and Trowell, who observed that several diseases typical for affluent societies were unusual in developing countries such as in East Africa^{1,2}. They then put forward the hypothesis that the Western diet, with a low content of dietary fibre, was connected with several disorders and diseases commonly found, such as constipation, diverticulosis, gallstones, atherosclerosis, diabetes mellitus and large-bowel cancer. However, dietary fibre is not a homogeneous substance, and different types of dietary fibre have different physiological effects.

During the last decade knowledge in the field of dietary fibre has increased considerably, and there is now experimental evidence supporting some of the hypotheses of Burkitt and Trowell. However, there are still many unanswered questions concerning the structure and physiological function of dietary fibre. In order to be able to correlate the structure of dietary fibre with physiological effects, well defined dietary fibre preparations, with respect to chemical composition, structure and physical properties are needed. It would also be a step forward if adequate methods for determination of the dietary fibre content and its components were used more generally.

Dietary fibre is by definition resistant to the digestive enzymes in the alimentary tract. However, it can be partly broken down by bacterial enzymes in the large intestine, and the physiological effects of dietary fibre are much dependent upon the extent of microbial degradation. The present dissertation presents data on the breakdown of different types of dietary fibre in the rat compared with man. The influence of parameters such as adaptation time to the diet, level of protein in the diet and particle size of the fibre on its fermentability have been studied as well as the effect of processing.

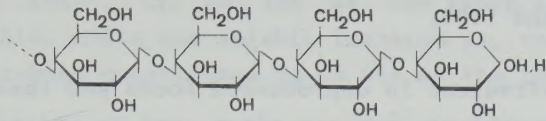
2.1 Definition

Trowell et al³ defined dietary fibre as the parts of the plant material that are not digested by the alimentary enzymes of man. This definition includes, apart from non-starch polysaccharides and lignin, other substances associated with the cell wall such as tannins, cutin, waxes and proteins. However, it is still a matter of discussion whether these cell-wall associated substances should be included in the definition of dietary fibre. Most workers prefer to include all non-starch polysaccharides and lignin, but not other cell-wall components, in the dietary fibre concept⁴⁻⁶.

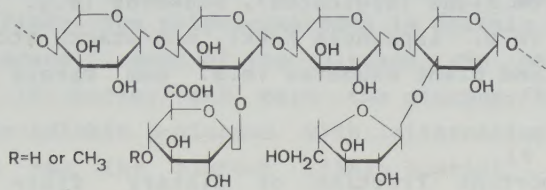
2.2 Composition and conformation

The quantitatively most important fraction of the dietary fibre is the polysaccharides, which differ in water-solubility as well as in molecular weight. Consequently, different types of polysaccharides can be expected to have different physiological effects.

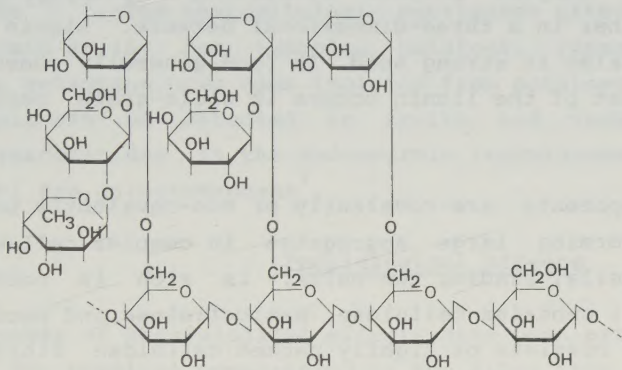
Extensive reviews on dietary fibre composition have been published⁷⁻⁹. The fibre polysaccharides are usually classified into cellulose, hemicelluloses and pectic substances. Cellulose is the most abundant polysaccharide in plant material. The molecule is a linear polymer, consisting of β -1,4-linked glucose units (Fig. 1). It contains up to 10 000 monomers, and is insoluble in water. Hemicellulosic polysaccharides (i.e. non-starch polysaccharides except cellulose and pectin) are highly branched polymers containing pentose and hexose sugars. The chemical composition and the solubility of the hemicellulosic substances vary widely. The monosaccharide found in the backbone in most cereal hemicelluloses is xylose (Fig. 1). Other monomers of the backbone are, for example, glucose (dicotyledons)⁷ and mannose (e.g. in palm seed)⁸. The side-chains in the hemicellulosic molecules often contain arabinose, galactose and glucuronic acid. The degree of polymerization is usually between 50 and 200. Pectic substances, which are most abundant in fruits and vegetables, are water-soluble polymers. The backbone often contains rhamnogalacturonans, where the uronic acids can be partly esterified (Fig. 1). Neutral sugars (e.g. arabinose, xylose and glucose) and glucuronic acid



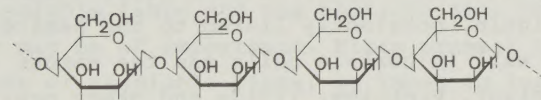
Cellulose



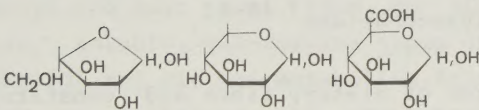
Arabinoxylan



Xyloglucan



Mannan



Rhamnogalacturonan

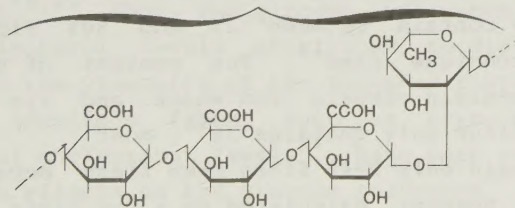


Fig.1 Examples of polysaccharides in dietary fibre

are present in the side-chains. The number of monomeric units has been reported to be between 150 and 1500.

Other polysaccharides are infrequent in unprocessed foods and therefore of less quantitative importance, but are often used as additives. These are polysaccharides from algae (alginates), seaweeds (e.g. carrageenan), seed-mucilages (e.g. ispaghula husk), non-starch storage polysaccharides (e.g. guar) and plant exudates (e.g. gum karaya and gum arabic).

Another quantitatively important fraction of dietary fibre is lignin^{7,8}. This is a complex polymer consisting of phenylpropane units linked to each other in a three-dimensional network. Lignin is insoluble in water and also in strong acid. In food generally consumed in Western countries most of the lignin occurs in whole-grain cereals and bran.

The different fibre components are covalently or non-covalently bound to other molecules forming large aggregates in complex cell-wall structures^{7,10}. The lamella, binding the cells, is rich in pectin. The primary cell wall contains cellulose, hemicellulose and pectin. The secondary cell wall consists of tightly packed cellulose fibrils, held together with non-covalent bonds. The cellulose fibrils exist in a matrix of hemicellulose. Lignin, covalently linked to the hemicelluloses, infiltrates the whole wall in certain cells and the amount increases with the age of the plant. Proteins, lipids and other non-carbohydrate substances, such as phytic acid, can also be linked - covalently - or associated with the polysaccharides^{7,10-12}.

Cereal grains are an important source of dietary fibre and constitute more than 40% of the daily fibre intake in Sweden¹³. Bran is the richest source of fibre and may contain between 25 and 50% fibre, depending on the milling technique used¹⁴. The content of non-starch polysaccharides in whole-grain cereals from wheat and rye is 10-14%, whereas refined wheat flour only contains 3%¹⁵. Most fruits and leaf vegetables contain only 2-4% fibre when fresh because of their high water content. However, calculated on a dry basis the values are in the same range as whole-grain cereals and not far from the brans, 10 to 30%¹⁴. Fruits and vegetables contribute about 30% to the daily fibre intake, while potato provides a further 20%¹³.

The water-soluble fraction of the fibre in bran is small, usually less than 10%¹⁴. Up to 30% of the fibre in whole-grain cereals may be soluble, while the soluble fraction in refined cereals, fruits and vegetables constitutes up to 60% of the total fibre^{14,15}.

The chemical composition varies considerably between different types of fibre. The polysaccharides in cereals consist of arabinoxylans and β -glucans¹⁴. Most of the glucans can be classified as cellulose, but in barley and oats the glucans, to a great extent, consist of water-soluble β -glucans with alternating 1,4 and 1,3 linkages¹⁶. Bran has the highest lignin content¹⁴. In contrast to cereals, the polysaccharides in fruits and vegetables contain high levels of uronic acids^{14,15}. The hemicellulosic substances often consist of xylo-glucans as in, for example, potatoes, runner beans and apples⁷. Pure galactans have been isolated from potatoes. Only small amounts of lignin can be detected in fruits and vegetables¹⁴. The storage polysaccharides in the endospermic leguminouses (e.g. locust bean and guar) are galactomannans⁷.

2.3

Physiological effects

A number of physiological effects have been attributed to dietary fibre. The chemical composition of the fibre, the proportion of insoluble and soluble fibre and the particle size of the fibre are all factors that may be of importance. Fibre present in cereal brans, with a large amount of insoluble fibre, is known to increase the faecal bulk and reduce the transit time, while gel-forming types of fibre, such as pectin and guar gum, have no significant effects¹⁷⁻²⁰. In contrast, soluble, gel-forming types of dietary fibre seem to affect cholesterol and glucose metabolism favourably, whereas insoluble fibre, such as cellulose and wheat bran, have lesser effects in this respect^{21,22}. Thus, the glucose tolerance and the decrease in serum cholesterol levels attributed to dietary fibre seem to be connected with the viscosity of the ingested fibre. However, favourable effects of insoluble fibre, such as bran, on the high-density-lipoprotein (HDL) cholesterol levels²¹, have been reported as well as long-term effects on the glucose tolerance²². A larger particle size of the fibre also seems to be beneficial in connection with glucose metabolism²³.

Epidemiological observations have indicated an inverse relation between fibre intake, especially of pentosans, and the development of large-bowel cancer^{24,25}. It has been suggested that fibre might offer protection through its capacity to, increase faecal bulk and dilute the concentration of carcinogens in the large intestine, decrease the transit time and thus reduce the exposure of the mucosa to carcinogens, or change the enzyme activity of the colon microflora and thus the formation of carcinogens. Another possible mechanism may be changes in the faecal composition due to the fibre. It has been shown that the concentration of steroids and bile acids in faeces, some of them carcinogenic, is higher after a low-fibre diet²⁶.

There are also reports, that fibre, especially from bran, has an inhibitory effect on mineral absorption. However, it is still not known whether the decrease in mineral absorption is due to the fibre as such or to other substances associated with the fibre, for example phytic acid²⁷. Other units present in fibre and known to bind minerals, at least in vitro, are the uronic acids²⁸.

Other effects that are related to the dietary fibre intake, are an increased excretion of protein and fat, resulting in a decreased utilization of energy²⁹. The increase in faecal fat and protein can be due to decreased digestion³⁰ or to increased microbial synthesis of protein and fat^{31,32}.

Some of the physiological effects described above, especially those related to the large-bowel function, may be dependent upon the extent of fermentation of the fibre.

3.1 Definition

By definition, dietary fibre is resistant to the alimentary enzymes in the digestive tract of man⁴, that is enzymes in saliva, the stomach and the small intestine. The relevance of this theoretical definition has been confirmed through studies in ileostomy patients³³. However, in the large intestine dietary fibre is partly degraded by the microflora⁵. Since the breakdown of fibre by bacteria in the large intestine is anaerobic, this process should be referred to as fermentation³⁴.

3.2 Microbial activity in the colon

The large intestine of monogastric animals contains approximately 10^{11} micro-organisms per gram wet content, (i.e. a total weight of approximately 1 kg in adult man) whereas the stomach, duodenum and jejunum contain less than 10^4 micro-organisms per gram wet content³⁵⁻³⁷. Human faeces contain approximately 400 microbial species of at least 40 genera³⁸. The most common genus capable of fermenting plant polysaccharides contains species of Bacteroides³⁹. It is also assumed that most of the fermentation in the colon can be ascribed to this type of micro-organism³⁹. Most of the species in the colon are oxygen-intolerant and over 99% of the bacteria obtain their energy through anaerobic processes³⁸. Many of the micro-organisms in the human colon are saccharolytic, that is they require a fermentable carbohydrate for growth³⁹. Further, the saccharidases studied in the microflora of man up to now are produced only when the micro-organisms are exposed to the polysaccharide, that is they are inducible³⁹. To maintain the colonic microflora it is assumed that 30-45 g of fermentable carbohydrates are required daily³⁹. The mean intake of dietary fibre in Sweden is calculated to be about 15 to 20 g per day¹³. Calculations from other Western countries give similar results⁴⁰. The source of the rest of the carbohydrate, assumed to be required for maintenance of the intestinal flora, might be carbohydrates from the intestinal mucins but this is not proven³⁹. A vegetarian diet may contain about five times more fibre than a normal diet, and diets in the developing countries have been reported to provide up to 150 g dietary

fibre per day^{40,41}.

The major products formed during fermentation are short chain fatty acids (SCFA) and gases (H_2 , CO_2 and CH_4)³¹. The SCFA's (acetic, propionic and butyric acid) can be absorbed through the mucosa, metabolized by the microflora or excreted in the faeces (5-20 mmol in the faeces of man)⁴². As mentioned above the daily fibre intake is about 15 to 20 g^{13,40}. It has been suggested that 10 to 15 g are fermented, which results in at least 100 mmol of SCFA⁴². Thus about 80 mmol could be absorbed, yielding 100kJ (i.e. about 1% of the energy intake), or further utilized by the bacteria. The SCFA's are probably absorbed in their unionized form through the mucosal cells and therefore the SCFA absorption might affect the pH of the colon content and thus the transport of other ions³¹.

The chemical structure, the degree of lignification and the solubility of the fibre are factors that may influence the susceptibility to bacterial fermentation³⁴. Lignin and cellulose are known to be resistant to bacterial fermentation, whereas gel-forming types of dietary fibre are known to be easily fermented^{17,20,43,44}. The fermentability may also be influenced by a number of other factors, such as particle size, level in the diet, or by the type or degree of processing⁴⁵.

3.3 Physiological effects related to fermentation

Different types of dietary fibre are fermented to various degrees. The extent of fermentation can be dependent upon the solubility and the chemical structure of the dietary fibre^{17,20,43-45}. A high resistance towards fermentation is related to a high bulking capacity (i.e. the capacity to increase the volume of faeces), whereas easily fermented types of dietary fibre have a low bulking capacity⁴⁶. On the other hand, these types of fibre can be used as a source of energy by the colonic microflora for cell-growth and maintenance⁴⁷. The absorption of minerals and vitamins may also be influenced by the degree of fermentation. It has been suggested that calcium can be absorbed in the colon after being released from the fibre during fermentation²⁸. It has also been shown, in rats, that pectin, which is an easily fermented type of dietary fibre, increases the microbial activity in the colon and, as a consequence, the synthesis of niacin,

thiamin and riboflavin³². There are also hypotheses that dietary fibre might protect against large-bowel cancer²⁵. The resistant types of dietary fibre might offer protection through their capacity to increase the faecal bulk and thus reduce the concentration of carcinogenic compounds or their capacity to bind carcinogens. The easily fermented types of fibre might offer protection by changing the bacterial flora and its metabolic activity, for example by the induction of bacterial enzymes that either inhibit the formation of carcinogens or transform existing carcinogens into less active substances²⁵. Another positive effect may be the pH fall in the colon that might be connected with the increased production of short chain fatty acids after the passage of easily fermented types of fibre. It has been shown that patients with large-bowel cancer have higher faecal pH values than patients in a control group²⁵.

Many physiological processes in animals are also assumed to be influenced by the composition and metabolic activity of the colon microflora⁴⁸. Some diseases might be influenced by the absence of certain micro-organisms rather than by the presence of specific bacteria in the intestinal flora³⁵. The composition of the human faecal microflora seems to be stable in terms of species and relatively independent of diet³⁸. However, certain disturbances in the composition of the microflora have been shown after starvation and after the intake of antibacterial drugs⁴⁸.

4 OBJECTIVES OF THE INVESTIGATION

The aims of the present work were:

- To develop experimental conditions for a rat model that could be used to predict the extent of fermentation of dietary fibre in man. In order to study factors that could affect the bacterial fermentation, particle size, adaptation time and fibre and protein levels were varied.
- To study the fermentability of different types of dietary fibre in relation to chemical structure, solubility and degree of lignification.
- To study dietary fibre in cereals of different extraction rates concerning content, composition and fermentability.
- To study the fibre content and composition before and after processing (extrusion cooking and popping), and when an effect was observed in vitro, the fermentability.
- To compare the bulking effect and the fermentability of some bulk laxatives on the Swedish market.
- To compare the fermentation of dietary fibre in man and in rat.

5 METHODOLOGICAL CONSIDERATIONS

5.1 Food

Methods for quantitative analysis of dietary fibre can be divided into gravimetric methods and fractionation methods.

The crude fibre method includes extraction with dilute acid and alkali and a subsequent isolation of the fibre by filtration⁴⁹. Using this procedure only a small and variable part of the total fibre is collected. In cereals of different extraction rates the crude fibre method was shown to recover only 15 to 75% of the total dietary fibre (IV). No standard conversion factor can be used and nowadays the method has to a large extent been abandoned.

Detergent methods developed by Van Soest and co-workers⁵⁰ were originally developed for analysis of forage but their applications have also been extended to human food.

Neutral detergent fibre (NDF) is obtained after extraction with boiling sodium dodecyl sulphate (SDS), and the residue includes hemicellulose, cellulose and lignin. Soluble fibre components, such as pectins, are not determined with this procedure. Further, the neutral detergent system does not solubilise the starch efficiently, resulting in too high fibre values. Modified methods using amylase treatment are therefore used for starchy materials^{51,52}. The neutral detergent fibre method (with amylase incubation) applied to cereals was shown to correlate badly with the total fibre, whereas the correlation with insoluble fibre was fairly good (IV).

Acid detergent fibre (ADF) is the material remaining insoluble after treatment with cetyl trimethylammonium bromide in 1N H_2SO_4 , i.e. cellulose and lignin. The ADF fraction has been reported to contain pectins⁵³. The difference between NDF and ADF is the hemicellulose content.

Enzymatic methods include enzymatic digestion of protein and starch and recovery of the fibre by subsequent filtration. The previously used enzymatic methods measured only the insoluble fraction of the fibre⁵⁴⁻⁵⁶. However, modifications by precipitation of the soluble

polysaccharides with alcohol have been published recently⁵⁷⁻⁵⁹. A disadvantage with most enzymatic methods is the long incubation time (20 to 38 hours). In the enzymatic method of Asp and co-workers⁵⁹, measuring both soluble and insoluble dietary fibre, these problems have been overcome. The incubation time was reduced to 2x1 hour. Further the solubilisation of starch was improved in this method by including a thermostable α -amylase in the gelatinization step. The fibre residues have to be corrected for remaining protein and ash. The sum of the analysed polysaccharides and Klason lignin in cereals was shown to correlate well with total dietary fibre measured with the enzymatic method (IV).

After previous removal of starch and fractionation to separate the fibre components into cellulose, non-cellulose polysaccharides and lignin, colorimetric methods can be applied^{60,61}. The polysaccharides are measured colorimetrically with anthron for hexoses, orcinol for pentoses and carbazol for uronic acids. Lignin is determined as Klason lignin, that is the residue insoluble in 72% H_2SO_4 . The disadvantage here is the lack of specificity of colorimetric methods⁶².

Gas-liquid-chromatographic assays (GLC)^{14,15,63} are used for analyses of individual fibre components. First the dietary fibre has to be hydrolysed to its monomeric components. Thereafter, the neutral sugars of the fibre are derivatised mostly to their alditol acetates. Since the uronic acids are very resistant to hydrolysis and difficult to derivatise they are usually determined by a decarboxylation method¹⁴ or by colorimetric methods^{15,63}. Lignin, can be determined as Klason lignin or more specifically after oxidation with potassium permanganate⁵¹. The GLC methods used differ in many details regarding sample preparation, starch hydrolysis and corrections for acid hydrolysis losses.

It was necessary for the dietary fibre method used in the present work to include both soluble and insoluble fibre and also a detailed characterization of the dietary fibre constituents. Therefore dietary fibre was isolated using the method described by Asp and co-workers⁵⁹, and the dietary fibre was then further analysed with gas-liquid chromatography for the neutral sugars and with a decarboxylation method for the uronic acids¹⁴. The amount of residual

starch, measured as glucose after incubation with amyloglucosidase and Termamyl, was checked in the fibre residues. An advantage with the enzymatic gravimetric method as a preparation step, is that the standardization of the subsequent characterization of fibre constituents can be checked with the gravimetric value. Another advantage is that the fibre residue can be analysed for residual starch.

5.2

Faeces

The bacterial mass contained in human faeces is assumed to constitute at least 30% of the total faecal weight³⁹. There is a close relation, between bacteria and fibre constituents, since they both contain polysaccharides. Hexoses, uronic acids, and amino sugars are common in bacterial saccharides, and hexoses, pentoses and uronic acids in dietary fibre^{8,64-66}. Thus, it is difficult to distinguish unfermented fibre polysaccharides from polysaccharides of bacterial origin. Few studies have been carried out to determine to what extent the saccharides excreted in the faeces can be bacterial cell-wall polysaccharides. A gravimetric technique, however, for quantifying the total amount of bacteria was developed by Stephen and Cummings⁴⁶, but no further characterization of the bacterial saccharides was made.

The intestinal mucin production by the host itself is another contributing factor. Intestinal mucin contains high concentrations of glycoproteins, consisting of hexoses, hexosamines and sialic acids⁶⁷. The intestinal mucosa is replaced every fourth to fifth day⁶⁸. However, mucin and epithelial cells have not been detected in faeces of animals with bacterial flora, whereas considerable amounts have been reported in the faeces of germ-free rats⁶⁹.

The balance technique used in the present work, does not distinguish between fibre polysaccharides, bacterial polysaccharides or endogenous polysaccharides. However, the low levels of saccharides detected on a starch based diet with no added fibre indicate that basal excretions of bacterial and endogenous saccharides are small(I). However, it cannot be excluded that the excretion of these saccharides might increase with the source and level of fibre present in the diet. On the other hand, guar gum, which might be expected to stimulate microbial activity in the large intestine, through its considerable fermentability, did not significantly increase the proportion of faecal saccharides

compared with that found with a fibre-free diet (I). In addition only saccharides present in the fibre preparations were detected in considerable amounts in the faeces (I). This strongly suggests that the excretion of bacterial and endogenous saccharides is comparatively small. However, to minimize this source of error the dietary fibre constituents excreted in faeces were corrected for saccharides appearing in faeces when the rats were fed a fibre-free diet (approximately 120mg/5d). In addition, the glucan values were corrected for the free glucose found in the faeces (<10mg/5d).

Another factor that might have an effect, is a further breakdown of the fibre by bacteria in the faeces between the time of excretion and collection. In the present work, the faeces were removed every day and stored in a freezer at -20°C . However, since most of the bacteria in the colon are anaerobic (99%)³⁸ any further breakdown in an aerobic environment can probably be neglected. Furthermore, the fibre is exposed to bacterial attack at 37°C for 10 to 20 hours in the colon⁴³, and these conditions should be considerably more favourable for bacterial enzymes, than those in faeces awaiting collection. In addition the moisture content of rat faeces is quite low (approximately 30%), and experiments on pigs with high faecal moisture (70%), did not show any significant differences in the faecal amino-acid composition after storage up to seven days at low temperature⁷⁰, indicating that the bacterial activity in faeces is low. Thus, any further breakdown of the fibre excreted in faeces awaiting collection seems unlikely.

Still another factor, is whether young rats, used in the experiments, have a fully developed microflora. However, according to Savage³⁶ anaerobic bacteria can be detected in the large intestine immediately after feeding the animals solid food. The existence of an effective microflora is also reflected by the fact that only minor amounts of saccharides were detected in the faeces after guar gum was given (I).

This rat model seems to be useful for estimating the degree of fermentation of different types of fibre. It could be argued that the digestive tract in the rat differs from that of man. However, the primary interest is not to determine the extent of fermentation of different fibres in absolute figures, but rather to rank and compare their fermentabilities. The experimental conditions in this rat model are

strictly controlled, and this may be difficult or even impossible to achieve in balance experiments with man, for example when studying the effects of processing. Further, balance experiments in man are in fact also very artificial.

The following conditions were used in the balance experiments.

Male Sprague Dawley rats weighing approximately 80 g were divided into groups of five and kept individually in metabolic cages in a room maintained at 23°C with 50-60% relative humidity. Food intake was restricted to 10 grams dry weight per day and water was provided ad lib. After a four-day adaptation period, feed residues and faeces were collected during a five-day balance period. The faeces were lyophilized, weighed, milled and stored at -20°C until analysed.

In all experiments a group fed a basal diet, with no added fibre, was included (I). Casein, 10% was used as a source of protein and maize starch was the main carbohydrate. In experiments with refined dietary fibre the fibre preparations were added at the expense of starch. When investigating fibre from cereal flours, the flour was used as starch and protein source.

The total dietary fibre content (sum of soluble and insoluble constituents) in feed was isolated with the enzymatic gravimetric method of Asp and co-workers⁵⁹.

Dietary fibre composition in feed and in faeces was determined with gas-liquid chromatography (GLC) of neutral sugars as their alditol acetates¹⁴. Allose was used as an internal standard. The samples were hydrolysed in 12M H₂SO₄ for 2 hours and then diluted to 0.36M and reflux-boiled for 6 hours. Lignin was measured as Klason lignin, that is the residue insoluble in 12M H₂SO₄. GLC was carried out on a Varian 3700 gas chromatograph equipped with a flame ionisation detector and a glass column (0.2 cm x 180 cm) packed with 3% SP-2340 on Supelcoport (100/120 mesh) at 180-225°C and a carrier gas flow of 30 ml/min, or with a WCOT fused silica capillary column (0.24 mm x 12.5 m, Cp Sil 19 CB, Chrompack) at 180-215°C and a carrier gas flow of 1.50 ml/min. The peak areas were measured with a Hewlett Packard integrator (3380-S). Corrections for hydrolytic losses and detector response were made by running the analysis with known sugar standards.

Uronic acids were determined with a decarboxylation method developed by Bylund and Donetzhuber⁷¹. The apparatus, obtained from Billerud AB (Säffle Sweden), contained a round bottom flask with heating device, a washing flask, and a thermostat-controlled absorption cell with electrodes for conductivity measurements. The changes in conductivity were registered continuously with a potentiometric recorder. Experimental details were the same as those described by Theander and Åman¹⁴.

Klason lignin and uronic acids were determined directly using the raw material. All dietary fibre saccharides were expressed as their polymer weight, i.e. monomer weight x 0.9. When analysing cereal fibre the fibre residues were checked for remaining in vitro resistant starch. The excretion of dietary fibre constituents in faeces was corrected for typical fibre saccharides excreted after a fibre-free diet (approximately 120 mg/5d). In addition, glucose values in faeces were corrected for free glucose and, when relevant, for undigested and unfermented starch.

7.1 The influence of milling and processing on dietary fibre

The proportions of soluble and insoluble fibre, the chemical composition and the degree of lignification of the fibre may all be important factors affecting the resistance of fibre to bacterial fermentation in the large intestine.

In paper IV the variation in the proportions of soluble and insoluble fibre with respect to the rate of extraction in flour from wheat, rye, barley, sorghum, rice and maize was studied. Dietary fibre constituents were determined in flours having the lowest (approximately 65%) and the highest extraction rates (100%).

As expected, the dietary fibre content increased with the rate of extraction. The dietary fibre content of refined wheat flour (i.e. extraction rate of about 65%) was 20% of that in whole-grain flour, whereas refined rye, barley and maize flour contained about 40% of that in whole-grain flours. The soluble fibre fraction however was independent of the extraction rate, suggesting that the main source of soluble fibre is from the endosperm. The soluble fibre fraction in wheat, rye, barley and rice constituted a major part of the total fibre (about 50%) whereas the soluble fraction in maize and sorghum was small (5-15%).

The dietary fibre constituents arabinose, xylose and glucose could be detected in all the investigated cereals. The monomeric compositions of the fibre in refined and whole-grain flours of wheat, rye and maize were similar, suggesting a similar arabinose-xylose ratio in both endosperm and outer layers. Refined flours from barley, rice and sorghum mainly consisted of glucans. Whole-grain flours of barley and rice contained considerable amounts of xylans. This suggests that the husk of these cereals is rich in xylose. The polysaccharides isolated from the husk of oats have also been shown to contain high levels of xylose, 35% (M.Nyman and N.-G.Asp, unpublished results). The outer layers of sorghum contained arabinoxylans. Considerable amounts of lignin were found only in the whole-grain flours.

A critical step in all dietary fibre determinations, is the starch degradation. Remaining starch adds to the dietary fibre and appears as glucose in the gas-chromatographic determinations. In unprocessed products the problem seems to be negligible and only small amounts of starch have been found in the fibre residues (IV)¹⁵. However, thermal processing can render a fraction of the starch less available to enzymes and thus increase the dietary fibre value⁷². Even if only a small fraction of the starch in products rich in starch remains enzyme-resistant during processing this would add to the dietary fibre value.

Paper VI reports on the importance of the enzyme system used for solubilisation of starch before and after extrusion cooking, in relation to dietary fibre values. When the heat-stable α -amylase Termamyl was excluded from the fibre assay, an increase in fibre content of up to 6% was seen after extrusion cooking. However, when Termamyl was included the increase in the fibre value was reduced to 1%. The differences in fibre values were directly related to the glucan content in the fibre residues. The considerable increase in glucan content could partly be due to the formation of amylose-lipid complexes after extrusion cooking. Amylose-lipid complexes are less readily degraded by pancreatic α -amylase at 37°C, but efficiently hydrolysed by Termamyl at about 100°C⁷³. Physiological amylose-lipid complexes should also be regarded as starch rather than dietary fibre as they are completely digested in vivo⁷³.

Solubilisation of insoluble fibre could be observed after extrusion cooking, especially in refined wheat flour samples. The solubilisation was less pronounced in products processed at a higher feed rate. The increase in soluble fibre could be due to the breaking of glycosidic bonds during processes that involve shear, as seen in cellulose during dry-ball milling⁷⁴ or in starch during extrusion⁷⁵.

7.2 Dietary fibre fermentation in the rat intestinal tract

7.2.1 Effect of adaptation time and protein level

The choice of adaptation time and level of protein in the diet are factors that could influence the fermentability of fibre. Regarding the adaptation time, dietary fibre might modify the microbial flora in the large intestine by induction of bacterial enzymes which will increase the fermentability of the fibre. However, none of the studies in the literature indicates that fibre is broken down more extensively with increasing adaptation time. No adaptation of the faecal microflora to ferment the fibre was observed in man or pigs by Ehle et al⁷⁶. Cunningham et al⁷⁷ reported similar fermentability of a resistant type of fibre (solkaflor) in pigs after different feeding times. Further, Cummings et al did not find any change in the fermentability of wheat bran fibre in man after three or six weeks⁴⁵.

In the present rat model a four-day adaptation period and a protein level of 10% in the diet were used. To ascertain if these parameters could be limiting for the fermentative breakdown of the dietary fibre, the level of protein in the diet was varied and the adaptation time to the diet prolonged. One resistant type (wheat bran) and one easily fermented type of fibre (low-methoxyl pectin) were investigated (II).

Prolongation of the adaptation period, from four days to eighteen days, decreased the faecal dry weight and in the case of wheat bran the decrease was significant ($p < 0.01$). The amount of dietary fibre excreted, however, was similar, while the nitrogen excretion, and probably also fat, decreased ($p < 0.01$) with increasing adaptation time. A tendency towards a decreased variation between individual rats, in faecal dry weights and in excretion of uronic acids after low-methoxyl pectin could also be seen as the adaptation period was extended. Thus, a longer adaptation period had no pronounced effects on the fermentability of a resistant type of fibre or on an easily fermented one, and a four-day adaptation to the diet seems to be adequate when evaluating the fermentability of fibre in rats.

With a protein level of less than 5% in the diet, the fermentation of the fibre was significantly impaired (II). The main dietary fibre constituents showed a similar trend, and the protein level seemed to

be a limiting factor. A level of protein higher than 10% however, did not further increase the fermentability. Thus, a protein level of 10% seems to be appropriate.

7.2.2 Effect of fibre level

An increasing level of fibre in the diet might decrease the fermentability. Keys et al⁷⁸ and Frank et al⁷⁹ reported a reduced breakdown of fibre, with increasing fibre levels. In contrast to these findings, however, Kies et al⁸⁰ found that cellulose given at different levels was fermented to the same degree. Similar results were also found by Garrisson et al⁸¹. When feeding rats with different levels of bagasse, no significant effects on the fibre breakdown were detected.

In paper II the influence of fibre level on the degree of fermentation was studied. One resistant type of fibre (5 and 10% wheat bran fibre) and one easily fermented type of fibre (4 and 8% pectin fibre) were investigated. Similar amounts of the fibre, calculated as per cent of intake, were recovered in faeces at both levels after intake of both types of fibre. The faecal excretion of dietary fibre constituents was 61% after the intake of wheat bran versus approximately 25% after pectin. However, a tendency to a decreased faecal excretion of uronic acids after the pectin diet was detected at the lower fibre level as well as a decreased variation between individuals. Cellulose, given at two different levels, 0.7 and 2% respectively, was also shown to be fermented to similar extents, 20% (V). Thus it seems that certain glycosidic linkages in the polysaccharides are resistant to fermentation.

7.2.3 Effect of particle size

It has often been proposed, that the particle size of the fibre affects the susceptibility to bacterial fermentation, as well as the faecal wet and dry weights⁴⁵. In vitro studies have shown that the water-holding capacity increases with particle size⁸². This may also be of importance in vivo. It has been established that the faecal wet weight decreases, when the particle size of the fibre is reduced⁸³⁻⁸⁵. However, the connection between particle size and faecal dry weight or the availability to bacterial degradation is still not fully understood⁸⁴⁻⁸⁶. The degree of breakdown of cellu-

lose by cellulolytic bacteria has been shown to be proportional to the size of the particles⁸⁶. Coarse particles can therefore be considered to be less susceptible to the colon microflora than fine. Heller et al⁸⁵ concluded that cellulose from coarse bran was less fermented than that from fine bran in man. Ehle and co-workers⁸⁷ however, have reported contrary results with bran on pigs. They interpreted the results as being due to the longer transit time of the coarser particles. On the other hand, Van Dokkum et al⁸⁴ have found that the fibre of coarse and fine bran bread were fermented to similar degrees.

In paper II the faecal bulking effect and the fermentative breakdown of the fibre were investigated after the intake of bran of two particle sizes (<0.4mm or 0.4-1.4mm). Similar amounts of the total fibre and of the main fibre constituents were recovered in the faeces. The faecal recovery of the main components, arabinose, xylose and glucose was 69, 46 and 65%, respectively, after milled bran, versus 70, 43 and 70% after the administration of coarse bran. The faecal dry weights obtained after milled and coarse bran were also similar. However, no definite conclusions can be drawn concerning faecal wet weights in these rat experiments since the faeces may have dried somewhat between the time of excretion and collection.

Thus, the particle size per se did not affect the bacterial fermentability of the fibre in the experiments described in paper II. Similar results were also obtained in another study (VI). Extrusion cooking, which is a process leading to an increase in the surface area of particles, did not increase the availability to bacterial enzymes of the fibre in whole-grain wheat flour. The different effects obtained in various investigations after intake of fine and coarse fibre might be explained by the use of quite different particle sizes.

7.2.4 Effect of structure, solubility and lignification

Dietary fibre encompasses a heterogenous group of substances, and different dietary fibres are known to be fermented to quite differing degrees. As early as 1936, Williams and Olmsted⁸⁸ reported that dietary fibre from carrot and cabbage was more easily fermented than fibre from wheat bran. More recently, similar findings have been reported by other authors¹⁹.

Cellulose is known to be quite resistant to bacterial breakdown. The resistance of purified cellulose and cellulose in bran was reported to be higher than that of cellulose in fruit and vegetables^{29,19,88}. The resistance of cellulose in bran is assumed to be dependent upon the degree of lignification. Chandler et al⁸⁹ found that lignin impaired the in vitro rumen digestion of other cell-wall constituents. In contrast Bertrand et al⁴³ did not find any differences in the fermentability of ordinary and delignified wheat-bran fibre, in rats. Another important factor is the physical structure of the material. Van Soest⁹⁰ have shown that cellulose breakdown in ruminants is dependent upon its crystallinity.

Hemicellulosic and pectic substances are known to be more easily fermented than cellulose, probably due to the larger proportion of soluble fibre. Prynne and Southgate⁹¹ found that highly soluble ispaghula fibre was fermented to a great extent in man and Cummings et al²⁰ found that pectin was completely fermented. However, in contrast to these findings, Brown et al⁹² found that sterkulia gum, a polysaccharide with a very high water-holding capacity but rather insoluble, was recovered unfermented in the faeces of rats. The importance of the proportion of soluble fibre was further demonstrated by Salyers et al⁹³. An insoluble and unfermentable xylan from Larchwood was degraded by bacterial enzymes of the genus Bacteroides when a fraction of the polysaccharide was solubilised by autoclaving. If the insoluble part was further solubilised, it was again degraded to the same extent.

Lignin is known to be completely resistant to bacterial fermentation. However, lignin is mostly determined gravimetrically as Klason lignin, which is a crude method not able to detect small differences accurately. The degradation of lignin in the anaerobic environment of the colon can probably be neglected. As far lignin has been reported to be degraded mostly by fungi, acting preferably in aerobic environments⁹⁴.

The presence of other fibre components, for example the addition of an isolated fibre such as cellulose, in the diet might also change the fermentability. Van Soest and co-workers have shown that a previous intake of pure cellulose reduces the breakdown of other fibre components in a subsequent diet⁹⁵.

The present work shows that dietary fibres from bran and whole-grain cereals are relatively resistant to fermentative breakdown, whereas fibres from fruits, vegetables and refined flours are more easily fermented (I, III and V). Of the fibre in wheat bran, about 50% was excreted in faeces. Guar gum, on the other hand, was almost completely fermented, while the faecal recovery of the uronic acids in pectin was about 20% (I). These types of fibre are completely different with respect to their chemical structure (monomeric composition, glycosidic linkages and polymerization) and to their physical properties (solubility, degree of lignification, distribution in the cells and three-dimensional structure) which may be of more or less importance for the degree of fermentation. Further, wheat bran, guar gum and pectin are all refined types of fibre which may also have an effect.

The various components of the sugar-beet fibre preparation, which contains similar amounts of arabinose-based hemicellulose, glucans (cellulose) and uronic acids (pectin), were fermented to quite differing degrees (I). Arabinose was completely fermented as guar gum, the uronic acids to a similar extent as those in purified pectins, and the glucans to a similar extent as that of wheat-bran fibre. These findings provide evidence that the chemical structure is of great importance for the degree of fermentation and that certain chemical linkages in the fibre are resistant to bacterial enzymes. This is further established by the fact that sterkulia, a gum with a very high water-holding capacity but rather insoluble, was almost completely resistant to fermentation (VII).

However, some physical properties also seem to be of importance for the degree of fermentation. The relative compositions of the fibre saccharides, in refined and whole-grain flours of wheat, rye and maize were similar in the endosperm and outer layers (IV), but the fermentability of the fibre in refined flours was considerably higher than that in whole-grain flours (V). Thus, fibres associated with the endosperm were more available to fermentation by bacteria. Some characteristic differences between the fibre in the outer layers and those from the endosperm, are that the soluble fibre originates mostly from the endosperm, whereas most of the lignin occurs in the outer layers (IV). This indicates that both the soluble fraction and the degree of lignification are of importance for the susceptibility to bacterial fermentation. However, the dietary fibres in refined rice,

barley and sorghum flours, consisting mainly of non-lignified glucans, were soluble to quite different degrees (IV). Fibre glucans with a small proportion of soluble fibre (sorghum) showed a similar resistance to bacterial fermentation as pure cellulose, 80% being excreted in faeces, whereas fibre glucans with a larger proportion of soluble fibre (barley and rice) were more extensively degraded, and less than 20% was recovered in faeces (V). These findings suggest that the proportion of soluble fibre is of significant importance for the fermentability.

The results of the present study indicate that the lignin content seems to be of less importance. Fibre from maize bran with similar dietary fibre content and similar proportions of fibre saccharides as wheat bran (II), but with considerably lower lignin content (2% in maize bran versus 7% in wheat bran), was more resistant to bacterial fermentation (M.Nyman & N.-G.Asp, unpublished results). The faecal recovery of the maize-bran fibre was 78% versus 61% of wheat-bran fibre. As the soluble fibre fraction in bran of both wheat and maize is small, this difference in fermentability indicates that other factors may also inhibit bacterial action, for example, antinutritional substances. This might further be suggested by the fact that different types of whole-grain wheat flours with similar fibre contents and compositions are fermented to quite varying degrees. Of total neutral fibre saccharides 56, 42 and 73% were recovered in faeces (V, VI and in 7.2.5). These differences are not connected with a low reproducibility, since the faecal recovery of the same source of pectin was similar at different occasions, 19-27% (I and II).

No interaction between dietary fibre constituents and fermentability was seen. The fermentability of fibre from refined flour was essentially the same with and without additional cellulose in the diet (V). The uronic acids of citrus pectin, which make up part of the fibre in ACO fibre tablets, were fermented to a similar extent as those of pure pectin (I and VII).

In conclusion, it seems that certain chemical bonds in dietary fibre are resistant to bacterial enzymes. An increasing amount of soluble fibre will slightly increase the availability to bacterial fermentation. The presence of lignin, or other dietary fibre components, seems to be of less importance for the fermentability. The mechanism behind

bacterial enzyme production, and hence the degree of fermentation is not, however, fully understood. Other factors might also be of importance, for example the presence of antinutritional substances. This field needs further investigation.

7.2.5 Effect of processing

The bulking effect of bran has been shown to be less pronounced with cooked than with raw bran⁹⁶. However, very few other studies have been made on dietary fibre with respect to processing, despite the fact that there are many factors in food processing that could be expected to have an effect on the fibre. Food processes that increase the surface area of a product might increase the susceptibility to fermentation. Berg et al⁸⁶ found that the breakdown of cellulose by cellulolytic bacteria was dependent upon the surface area. The importance of processing was further demonstrated by Johansson et al⁹⁷ and Varo et al⁷² who found that thermal processing can make a fraction of the starch less available to enzymes and thus increase the apparent fibre content. Further, degradation of glycosidic bonds in cellulose have been reported in processes that involve shear⁷⁴.

Extrusion cooking is a process where the raw material is exposed to intense mechanical treatment⁹⁸. The cooking is continuous and takes place at high temperatures (up to 250°C) and high pressures (2-20MPa), during short times (5-10s) and with a low water content (>5%). The mechanical treatment leads to a complete disorganization of the original structure. Popping on the other hand is a discontinuous process, where the material is subjected to elevated pressure (3-14 atmospheres) and temperature (240°C), and allowed to expand into atmospheric pressure during a longer period (10-15 min) and with no additional water.

Dietary fibre components in refined wheat flour tended to be more fermented after extrusion cooking than in the raw state. This process increased the fermentability of the fibre saccharides even with mild cooking conditions (VI). The faecal excretion of the main fibre components arabinose, xylose and glucose was, on average 22%, in raw wheat flour versus 12% after processing. However, the fermentability of the dietary fibre components in whole-grain wheat flour was similar before

and after extrusion cooking, about 50%. Thus, extrusion cooking only seems to have an effect on the fermentability of the fibre found in the endosperm. The considerable solubilisation of the fibre after extrusion cooking could be a factor that increases the availability to microbial degradation. However, the increased fermentability probably does not have any nutritional consequences, since refined wheat flour in itself has a low fibre content.

Dietary fibre content and composition in raw and popped whole-grain wheat flour are shown in Table 1. A redistribution of soluble and insoluble fibre could be observed with severe conditions (14 atmospheres). In raw flour 13% of the fibre was soluble while in popped flour 34% was soluble. During popping under severe conditions (14 atmospheres) the outer layers are partly removed from the wheat kernel, explaining the reduced dietary fibre content. The different main fibre components decreased by approximately the same level. There was no indication of modification of starch, as the glucose content did not increase.

Since the outer layers are partly removed during popping one would expect the lignin content to decrease after processing. However, the amount of lignin was similar in both raw and popped products. This could be due to the formation of lignin-like material, for example Maillard products, during the process, which add to the lignin content. The formation of Maillard products was further established by the fact that nitrogen was found in the lignin fraction after popping.

There was a tendency towards an increased fermentation of the dietary fibre saccharides arabinose and xylose after popping, but it was only significant ($p < 0.05$) in the case of xylose (Table 1). The lignin-like material disappeared to a larger extent after processing ($p < 0.01$). This can be due to the fact that the lignin-like material formed is more easily fermented during its passage through the gut.

Table 1. Composition and faecal recovery of dietary fibre in raw and popped whole-grain wheat flour.

	Composition of dietary fibre (% of dry matter)		Faecal excretion (% of intake)			
	Raw	Popped	Raw		Popped	
			Mean	SD	Mean	SD
Rhamnose	-	-	-	-	-	-
Arabinose	2.3	1.4	86	4	79	8
Xylose	3.8	2.7	56	8	44	4*
Mannose	0.2	0.2	92	39	62	11
Galactose	0.4	0.3	106	15	74	7
Glucose	3.2	2.3	74	9	76	8
Lignin	2.3	2.5	86	11	42	18**
Total	12.2	9.4	74	7	58	8**

** p<0.01, * p<0.05. Significantly different from the raw material (Student's t-test).

7.2.6 Effect of starch

It has been known for a long time that raw potato starch has a low digestibility⁹⁹. In contrast, cooked potato starch and other types of starch are considered to be completely digested and absorbed. However, recently the digestibility of starch in man has been a matter of much discussion, and there are suggestions that a considerable fraction of the starch might not be digested. It is assumed that to maintain the colonic microflora a carbohydrate intake of 30 to 45g is needed³⁹. With the exception of endogenous polysaccharides the daily supply of fermentable polysaccharides to the large intestine is only about 15g⁴², indicating the need for another fermentable carbohydrate than dietary fibre, for example undigested and unabsorbed starch. Anderson et al¹⁰⁰ proposed that as much as 10-20% of the carbohydrates in bread and pasta were not digested and absorbed. In addition, Stephen et al¹⁰¹ proposed that in average 10% of ingested starch, after a meal with a normal starch level, reached the large intestine undegraded. However, no faecal starch could be detected in rats by Björck et al¹⁰² after the intake of bread, even after elimination of the colonic microflora by antibiotic treatment. On the other hand resistant starch, i.e. starch available to amylase after solubilisation in 2M KOH, was nearly completely recovered in faeces in animals receiving antibiotics¹⁰².

Generally, only a very small amount, or no starch at all, was detected in faeces in the present work. However, a considerable amount of α -glucans (up to 330mg/5d) available to amyloglucosidase was detected in faeces when rats were fed refined wheat and sorghum flours or whole-grain wheat, sorghum and rye flours (V). With refined wheat flour 61% of the excreted glucans consisted of starch. It is important to bear this in mind when calculating the fermentability of the β -glucans. However, in relation to the starch intake the excretion of faecal starch was of no nutritional significance since it represented less than 1% of the starch ingested. On the other hand it cannot be excluded that a much larger fraction of the starch may have reached the colon.

7.2.7 Effect of antibiotic treatment

A study has been started to establish whether non-bacterial degradation of the fibre could occur in the rat intestinal tract.

Dietary fibre excretion with refined flour from hard winter wheat (extraction rate 69%) was studied with and without antibiotics (0.7% Nebacitin) in the diet (M.Nyman, I.Björck, B.Pedersen, B.O.Eggum and N.-G.Asp, unpublished results). Antibiotic treatment with Nebacitin on this level has been reported to eliminate the microbial activity in the colon to approximately 80%¹⁰³. Nebacitin consists of one third Neomycinsulphate and two thirds Bacitracin. Neomycinsulphate is an oligosaccharide consisting of amino sugars and ribose and Bacitracin is a mixture of polypeptides, thus containing no saccharides that could add to the fibre saccharides.

In rats fed diets without antibiotics the excretion of the main fibre components arabinose, xylose and glucose was 15, 16 and 36%, respectively (Table 2), and thus similar to those reported previously for refined flour from hard winter wheat (V). A considerable increase in the faecal excretion of saccharides could be observed after antibiotic treatment. The faecal recovery of the main fibre constituents seemed to be complete or nearly complete. However, the excretion of hexoses must be interpreted with caution. After a few days rats treated with antibiotics show characteristics similar to those of germ-free animals, which are known to excrete considerable amounts of mucins and epithelial cells, i.e. glycoconjugates consisting of hexoses, hexosamines and sialic acids^{35,69}. In the present study there was a four-fold excretion of, for example, galactose compared with the intake. Thus any possible effects of antibiotic treatment on the fibre glucose should be interpreted with care. Pentoses, however, have not been reported to occur in intestinal mucins and epithelial cells, and the excretion of these sugars could be suggested to originate from the fibre. In three of the rats there was a complete recovery of the fibre constituents in faeces, and in the other two the faecal recovery averaged 71%.

This strongly suggests that most of the fibre degradation, at least for arabinoxylans, occurs through fermentation.

Table 2. Composition and faecal recovery of fibre saccharides after refined wheat flour without and with antibiotic treatment.

	Composition of dietary fibre (% of dry matter)	Intake		Faecal excretion						% of intake			
		Mean		Without		With		mg/5d		Without		With	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Rhamnose	0.1	36	0	18	5	48	10	52	14	134	26		
Arabinose	0.9	300	0	44	4	270	55	15	1	91	18		
Xylose	1.1	380	0	56	5	350	69	16	2	91	22		
Mannose	0.2	59	0	14	7	87	21	25	11	147	35		
Galactose	0.2	80	0	51	11	230	64	62	14	281	78		
Glucose	0.9	300	0	110	10	310	95	36	3	111	32		
Total	3.4	1160	0	290	36	1300	305	25	3	114	26		

7.2.8 Fibre as bulk laxatives

Bulk laxatives are widely used in Western countries to avoid constipation and are often recommended instead of irritating laxatives. The laxatives are based on more or less refined fibre preparations. The dietary fibre source, however, varies and different types might therefore have different bulking capacities. Prynne and Southgate⁹¹ found that fibre from ispaghula was fermented to a great extent in man, whereas Eastwood and co-workers¹⁰⁴ found that sterkulia gum was resistant to fermentation.

Paper VII presents data on the bulking capacity as well as the faecal recovery of fibre, fat, protein and minerals in rats after the administration of four quite different bulk laxatives frequently used in Sweden; ACO fibre tablets (barley and citrus pulp), Fiberform (enriched wheat bran), Inolaxol (sterkulia gum) and Vi-Siblin (ispaghula husk).

The dietary fibre contents of the various bulk laxatives were quite different and varied between 45 and 82% (dry weight basis). However, bulk laxatives caused a similar increase in faecal dry weight per gram added fibre. In contrast, the water-holding capacity of faeces, measured in vitro, was considerably higher with Inolaxol and Vi-Siblin, than with the two other types (4-6 times). Compared with ordinary wheat bran (III), the faecal dry weights were somewhat higher after the intake of the bulk laxatives, but compared with maize bran they were similar (M.Nyman and N.-G.Asp, unpublished results).

Approximately 70% of the fibre passed undegraded through the gastrointestinal tract after the intake of ACO fibre tablets, Fiberform and Vi-Siblin, while Inolaxol was almost completely resistant to fermentation. The faecal excretion of nitrogen was similar for all the bulk laxatives studied, while Vi-Siblin caused a higher excretion of lipids than the others. The increment in dry weight resulting from Inolaxol was significantly ($p < 0.001$) higher (on average 30%) than that of the others. This was mainly due to an increase in mineral excretion. The capacity of Inolaxol to bind minerals could be due to its uronic acid units, but is probably more related to the kaolin, used as constituent in Inolaxol. Mineral binding studies in vitro have shown that as much as 70% of added Cu was bound by Inolaxol, whereas only 15% was bound

by sterkulia gum alone¹⁰⁵. This effect needs further investigation in man.

It is concluded, that the bulking capacity was pronounced with all the bulk laxatives investigated and somewhat higher than that with ordinary wheat bran. However, it should be noted that the cost of the bulk laxatives is up to 30 times higher than that of wheat bran.

7.2.9 Comparison between man and rat

There is a need for an animal model that can be used to predict dietary fibre breakdown in man. There are differences between man and rat that might be of importance for fermentation, for example the bigger caecum in the rat. Further, the rat is a small animal compared with man, with a faster transit time through the gut, which might result in a lower degree of fermentation. However, there are also similarities between man and rat. Bacteria, which contain saccharidases, and normally found in the human colon are Bacteroides, Bifidobacterium, Eubacterium, Peptostreptococcus and Ruminococcus¹⁰⁶. Similar species have been reported in the colon microflora of adult mice³⁶, and are probably also present in the rat¹⁰⁷. Comparative studies on fibre breakdown in man and rat have been done by Van Soest et al¹⁰⁸, who concluded that the degree of fermentation of fibre in man was considerably higher than in rats, but lower than in pigs.

Paper III presents data on the fermentative breakdown of dietary fibre preparations from bran, apple, cabbage, carrot and guar gum in man and rats. The fermentative breakdown of the neutral fibre saccharides showed good correlation between man and rat ($r=0.97$). Wheat bran was the least fermented, whereas the fermentation of the fibre from apple, cabbage, carrot and guar gum was more complete. There was no indication of a more extensive fermentation of fibre in man; on the contrary bran seemed to be somewhat more resistant. The different dietary fibre constituents in each preparation were also fermented to similar extents in both species. The variation between individuals was more pronounced in man, especially with the easily fermented types of fibre, showing that experimental conditions are more easily controlled in an animal model.

A good correlation in faecal dry weights between man and rat was seen

($r=0.95$). Wheat bran had the best bulking capacity, while those of apple, cabbage, carrot and guar gum were less pronounced. There was an inverse relation between the amount of soluble fibre and an increase in faecal dry weight.

It is concluded, that the rat model applied in the present work can be used to predict dietary fibre fermentation and bulking capacity in man.

CONCLUSIONS

The selected experimental conditions (adaptation time and protein level) seem to be appropriate when evaluating the fermentation of dietary fibre in rats. Prolongation of the adaptation period decreased faecal dry weight, due to a decreased excretion of protein and fat, while the fermentation of the fibre was practically unchanged. A protein level of less than 5% in the diet reduced the fermentability of the fibre, but a protein level higher than 10% did not increase the fermentation of the fibre further. The fermentability was not affected by the level of fibre or by the particle size.

The fermentative breakdown of different types of fibre was quite different. The chemical structure and the proportion of soluble fibre seemed to be of great importance. Certain chemical bonds in the fibre were obviously resistant to bacterial enzymes, while an increasing amount of soluble fibre partly increased the availability to bacterial enzymes. The presence of lignin was of less importance.

Extrusion cooking of refined wheat flour with mild conditions and popping of whole-grain wheat flour with severe conditions tended to increase the fermentation of the fibre. This increased fermentability could be related to the increase in the proportion of soluble fibre after processing. No effects on the fermentability after extrusion of whole-grain flour could be observed.

Generally no starch was found in faeces. However, after some cereal grain diets a considerable amount of faecal starch could be detected. With one type of wheat flour 61% of the excreted glucans consisted of undigested and unfermented starch, indicating a much larger fraction reaching the colon. In relation to the starch intake, however, the excretion represented less than 1%, indicating a very complete digestibility of starch.

The faecal recovery of dietary fibre from refined wheat flour was studied before and after antibiotics were included in the diet. The antibiotic treatment indicated an increased excretion of mucins and epithelial cells. The faecal recovery of the ingested fibre constituents arabinose and xylose was nearly complete. This strongly supports the theory that dietary fibre degradation in the rat intestinal

tract occurs almost exclusively by fermentation.

The dietary fibre contents of various bulk laxatives on the Swedish market were quite different. The laxatives however caused a similar increase in faecal dry weight per gram ingested fibre. Compared with ordinary wheat bran the bulking effect was somewhat higher with the laxatives, while the cost of the laxatives was considerably higher.

It is concluded that this experimental model is useful for the prediction of fermentative breakdown and bulking capacity of dietary fibre in man. Bran, apple, cabbage, carrot and guar gum were fermented to a similar extent in man and rat. A good correlation in faecal bulking capacity between rat and man was also seen.

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Fermentation of dietary fibre components in the rat intestinal tract

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1. The fermentative breakdown of dietary fibre from various sources in the intestinal tract was studied using rat balance experiments and gas-liquid chromatographic analysis of dietary fibre monomers in feed and faeces.

2. On a basal diet with 690 g maize starch/kg but no added fibre, small but detectable amounts of polymeric glucose, rhamnose, arabinose, xylose, galactose, mannose and uronic acids, i.e. sugars occurring in dietary fibre, were excreted in faeces.

3. Dietary fibre in wheat bran was rather resistant to fermentation; 63% was recovered in the faeces. Guar gum, on the other hand, was almost completely fermented, whereas 19 and 25% of the uronic acids in low and high methoxylated pectin respectively, were excreted in faeces. The various constituents of sugar-beet dietary fibre (approximately equal amounts of arabinose-based hemicellulose, pectin and non-starch glucan (cellulose)) showed quite variable availability for micro-organisms in that 6–12% of the arabinose, 17–25% of the uronic acids, and 52–58% of the cellulose were recovered in the faeces.

4. Faecal nitrogen excretion increased on addition of any one of the dietary fibre preparations studied, resulting in decreased true and apparent protein digestibility values.

5. The faecal dry weight increment was most pronounced when feeding bran and could then almost be accounted for by the remaining fibre and by protein. The less-prominent bulking effect of guar gum and pectins, that were much more extensively fermented, could be only partly explained by dietary fibre and protein.

Dietary fibre is by definition (Trowell *et al.* 1976) resistant to the digestive enzymes in the gastrointestinal tract. The microflora of the large intestine, however, is able to ferment partly the dietary fibre constituents. The extent of fermentation is quite variable between various types of dietary fibre. Thus lignin and also cellulose are highly resistant whereas pectin, guar gum and to some extent hemicelluloses are reported to be easily fermented. (Booth *et al.* 1963; Yang *et al.* 1969; Cummings *et al.* 1979; Dintzis *et al.* 1979; Gramstorff Fetzner *et al.* 1979; Heller *et al.* 1980).

The extent of fermentation of dietary fibre is important for its physiological effects. Thus, easily-fermented types of fibre, such as pectin, have poor bulking capacity. (Cummings *et al.* 1978). The very good bulking effect of wheat bran can be explained by its relative resistance to fermentation, leaving a substantial amount of water binding fibre also in the distal colon.

Since energy substrate is the limiting factor in colonic bacterial growth (Mason & Palmer, 1973) it is probably also important that a certain proportion of the dietary fibre is fermentable, thus providing energy for the bacterial growth. An increased bacterial mass contributes to the bulking effect of bran and is relatively more important for that of more easily-fermented types of fibre, for example cabbage (Stephen & Cummings, 1980).

It is still an open question whether fermentable dietary fibre can be utilized as a source of energy. Bond & Levitt (1976) have shown that lactic acid can be absorbed from the human colon, and Conrad *et al.* (1958) showed that ^{14}C from labelled soya-bean cellulose fed to rats was found in the expired carbon dioxide as well as in carcass and urine.

Inhibition of mineral absorption by dietary fibre can also be expected to depend partly on the extent of bacterial breakdown, since especially calcium might be absorbed from the colon if released there from binding dietary fibre (James *et al.* 1978).

The present study was undertaken to provide detailed information on the extent of

fermentation in the rat intestine of various dietary fibre constituents in wheat bran, guar gum, two pectins with different extents of methoxylation, and sugar-beet fibre. The effect of these types of dietary fibre on nitrogen absorption and utilization was also studied.

MATERIALS AND METHODS

Dietary fibre preparations

Coarse wheat bran (Kungsörnen, Sweden) was milled to particle size less than 0.4 mm. Guar gum and pectin with low (37%) and high (74%) extents of methoxylation were obtained from the Copenhagen Pectin Factory Ltd, Skensved, Denmark. Two dietary-fibre-rich preparations from sugar-beet pulp were obtained from the Swedish Sugar Company. These were also milled to a particle size less than 0.4 mm. The two preparations differed in that one of them (half soluble) was treated with hot water to increase the solubility of pectins.

Animals

Male Sprague-Dawley rats with initial weight 75–80 g were used. The rats were randomly divided into groups of five. They were kept individually in metabolism cages in an air-conditioned room maintained at 23° and 50–60% relative humidity. The food intake was restricted to 10 g dry weight/d. Water was provided *ad lib*. Following a 4 d adaptation period, feed residues, urine and faeces were collected during a 5 d balance period. Urine was collected in sulphuric acid (20 ml/l) and analysed for N. Faeces were removed every day and frozen at –20°. They were then lyophilized, weighed and milled to particle size less than 0.4 mm. N and carbohydrate analyses were then performed.

Diets

A basal diet was prepared as described in Table 1. Casein, 100 g/kg, was used as source of protein and maize starch was the main carbohydrate. No dietary fibre was included in the basal diet. In the experimental diets the dietary fibre preparations were substituted for maize starch with the following concentrations (g/kg): wheat bran 100, guar gum 100, high-methoxyl pectin 93, low-methoxyl pectin 94, sugar-beet fibre 100.

Analytic methods

Dietary fibre. The total dietary fibre content (sum of insoluble and soluble constituents) was assayed gravimetrically after digestion with physiological enzymes as described by N.-G. Asp, H. Hallmer, C.-G. Johansson and M. Siljeström (unpublished results).

Dietary fibre composition in the feed and in faeces was assayed by gas-liquid chromatographic determination of neutral sugars as their alditol acetates (Sawardeker *et al.* 1965). Uronic acids were determined using a decarboxylation method (Bylund & Donetzhuber, 1968). Lignin was determined as Klason lignin, i.e. the residue insoluble in H_2SO_4 (720 ml/l). Conditions for hydrolysis and other details were as described by Theander & Åman (1979).

All dietary fibre components are expressed as polysaccharides, i.e. values obtained with monosaccharides as standards are corrected by multiplication by the factor 0.9.

N determination. Food components, dietary fibre preparations, urine and faeces were analysed for total N by the Kjeldahl method using concentrated H_2SO_4 at 400° with selenium as catalyst (Tecator Kjeltex equipment). Crude protein was calculated as $\text{N} \times 6.25$.

Calculation of digestibility and biological value. True digestibility and biological value were calculated using the Thomas-Mitchell equations (Eggum, 1973). Correction factors for endogenous urinary N and metabolic faecal N were determined in separate experiments using 40 g egg protein and 50 g cellulose/kg diet. Apparent digestibility was calculated as $(\text{N}_i - \text{N}_f)/\text{N}_i$ where N_i is the N intake and N_f is the N in faeces.

Statistical evaluation. Student's *t* test (two-tailed) was used.

Table 1. *Components used in the basal diet mixture (g/kg)*

Component		Source
Casein ANRC 30 M	100	Humko Sheffield Chemicals, New York, USA
Sucrose	100	Swedish Sugar Company AB
Maize starch	692	AB Risenta, Sweden
Maize oil	50	Purchased through hospital pharmacy
Mineral mixture*	48	
Vitamin mixture†	8	
Choline chloride	2	

* Contained (g): $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 8.6, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 32.0, KH_2PO_4 7780, $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ 4024, CaCO_3 9600, KI 1.6, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 2000, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 180, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 80, CoCl_2 0.46, NaCl 2382.

† Contained (g): menadione 2.5, thiamin hydrochloride 10.0, riboflavin 10.0, pyridoxin hydrochloride 5.0, calcium pantothenate 25.0, nicotinic acid 25.0, folic acid 1.0, inositol 50.0, p-aminobenzoic acid 5.0, biotin 0.2, vitamin B_{12} , cyanocobalamin 0.015, vitamin A 0.86, vitamin D 0.025, vitamin E 100, wheat starch 3765.

RESULTS AND DISCUSSION

Dietary fibre composition and degradation in the rat intestinal tract

Basal diet. The maize starch used as the carbohydrate base in the diets contained 8 g dietary fibre/kg as measured by the enzymic gravimetric method described previously. Gas-chromatographic analysis of monomers after acid-hydrolysis of this fibre showed mainly glucose but also traces of rhamnose, arabinose, xylose, mannose and galactose. Thus, the small amount of dietary fibre in the maize starch was mainly in vitro indigestible starch or other glucans and to a smaller extent hemicellulose impurities.

Table 2 shows intake and faecal excretion of dietary fibre monomers on the basal diet containing 692 g maize starch/kg but no added dietary fibre. The faecal excretion was lower than the intake for most monomers but higher than the intake for rhamnose. A small amount of uronic acids was detected in the faeces but not in the diet.

It cannot be settled from our experiments whether the faecal excretion of dietary fibre monomers is due to remnants of the fibre in the maize starch resistant to bacterial degradation, endogenous polysaccharide excretion, or bacterial polysaccharide synthesis. The levels, however, were comparatively very small and therefore not essential to the interpretation of the further results, even if this basal excretion of dietary fibre monomers might vary with the diet.

Wheat bran. The wheat bran contained 498 g dietary fibre (moisture free basis)/kg as determined by the enzymic gravimetric method, and of this 27 g/kg was soluble. Table 3 shows the dietary fibre composition. As expected, the main monomer components were arabinose, xylose and glucose. Small amounts of mannose, galactose and uronic acids were detected as well. The Klason lignin content was 99 g/kg.

The faeces contained small amounts of rhamnose in addition to those present in the basal diet, as was the situation after feeding the basal diet. The faecal recovery of arabinose- and xylose-based fibre was 57 and 46% of the intake, respectively. These values are in good agreement with those reported by Bertrand *et al.* (1981). The glucose-based fibre, i.e. the non-starch glucans, seemed somewhat more resistant to fermentation, but after correction for the polyglucose excreted on the basal diet the extent of fermentation was similar to that of pentosans, as shown in Table 3.

Lignin seemed resistant to bacterial degradation, which is in agreement with earlier studies (Gordon, 1978; Bertrand *et al.* 1981).

Table 2. *Faecal excretion of carbohydrates by rats given the basal diet*

(Mean values and standard deviations)

	Intake of dietary fibre monomers (mg/5 d)*		Faecal excretion (mg/5 d)*	
	Mean	SD	Mean	SD
Rhamnose	3	0	12	4
Arabinose	10	1	5	3
Xylose	16	2	5	2
Mannose	20	2	4	1
Galactose	29	2	27	14
Glucose	168	19	68	27†
Uronic acids	—	—	4	1

* As anhydro sugars.

† Corrected for free glucose (approximately 3 mg/5 d).

Table 3. *Composition and faecal recovery of dietary fibre in wheat bran*

(Mean values and standard deviations)

	Composition of dietary fibre (g/kg dry matter)	Faecal excretion							
		Intake (mg/5 d)		mg/5 d		% of intake		Corrected for basal excretion	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Rhamnose	—	—	—	17	4				
Arabinose	99	489	2	279	22	57	4	57	4
Xylose	143	707	2	323	17	46	2	45	2
Mannose	4	18	0	13	1	72	7	50	7
Galactose	10	50	0	42	6	83	13	29	13
Glucose	131	647	2	395	30*	61	5	51	5
Uronic acids	5	25	0	14†		56		40	
Klasiin lignin‡	99	493	2	450	65	91	13		
Total	491	2430	8	1520	50	63	2	58	2

* Corrected for free glucose (approximately 3 mg/5 d).

† Analysed in one rat only.

‡ Residue insoluble in sulphuric acid (720 ml/l).

|| For details, see Table 2.

Wheat bran fibre was thus rather resistant to fermentation in the large bowel, 63% of the total fibre being recovered in the faeces. The bulking effect of bran can largely be explained by remaining dietary fibre. Thus, 75% of the faecal dry weight increment was due to remaining dietary fibre as shown in Table 10. This is in agreement with results from human studies (Stephen & Cummings, 1980).

Guar gum. This gel-forming type of dietary fibre is a rather pure galactomannan with (mmol/mol) 525 mannose and 311 galactose (Table 4). Our preparation also contained small amounts of arabinose, glucose and uronic acids.

The galactomannan of guar gum was fermented almost quantitatively. Thus, only approximately 1% of the mannose and 4% of the galactose appeared in the faeces. The minor components were also fermented almost completely.

Table 4. *Composition and faecal recovery of dietary fibre in guar gum*
(Mean values and standard deviations)

Composition of dietary fibre (g/kg dry matter)		Faecal excretion							
		Intake (mg/5 d)		mg/5 d		% of intake		Corrected for basal excretion†	
				Mean	SD	Mean	SD	Mean	SD
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Rhamnose	5	18	2	24	10	130	41	64	46
Arabinose	19	73	7	3	0.5	74	0.4	0	
Xylose	3	12	1	3	0.7	26	9	0	
Mannose	525	2070	190	18	12	1	0.5	0.6	0.5
Galactose	311	1230	110	48	21	4	1	2	1
Glucose	31	123	11	33	13*	26	10	0	
Uronic acids	15	59	6	9	5	15	8	8	8
Klason lignin	—	—	—	—	—	—	—	—	—
Total	909	3580	320	137	54	4	1	1	1

* Corrected for free glucose (approximately 3 mg/5 d).

† For details, see Table 2.

Table 5. *Composition and faecal recovery of dietary fibre in low-methoxyl pectin (extent of methoxylation 37%)*

(Mean values and standard deviations)

Composition of dietary fibre (g/kg dry matter)	Faecal excretion						
	Intake (mg/5 d)		mg/5 d†		% of intake		
	Mean	SD	Mean	SD	Mean	SD	
Rhamnose	7	26	4	14			
Arabinose	2	9	1	—			
Xylose	1	5	1	—			
Mannose	1	3	0	—			
Galactose	30	115	14	22			
Glucose	4	14	2	64*			
Uronic acids	873	3370	430	650	455	19	12

* Corrected for free glucose (approximately 3 mg/5 d).

† The aldoses are analysed in one rat only.

In spite of the almost complete fermentation of guar gum, this fibre had a certain bulking effect (Table 10). Obviously, this was due to increased faecal losses of non-fibre material (see p. 364).

Pectin. The low- and high-methoxyl preparations were rather pure uronic acid polymers with small amounts of rhamnose and galactose, and in the high-methoxyl pectin also arabinose (Tables 5 and 6). The mean faecal recovery of uronic acids was 19% for the low-methoxyl pectin and 25% for the high-methoxyl pectin. Thus, the low-methoxyl pectin seemed to be fermented more efficiently, which is not in agreement with the study of Gilmore (1965). However, in our study there was a considerable variation between different rats and therefore the difference was not statistically significant. The excretion of the minor dietary fibre monomers was approximately the same as on the basal diet.

Table 6. *Composition and faecal recovery of dietary fibre in high-methoxyl pectin (extent of methoxylation 74%)*

(Mean values and standard deviations)

	Composition of dietary fibre (g/kg dry matter)	Intake (mg/5 d)		Faecal excretion			
				mg/5 d†		% of intake	
		Mean	SD	Mean	SD	Mean	SD
Rhamnose	9	35	4	49			
Arabinose	35	137	15	—			
Xylose	2	9	1	5			
Mannose	1	3	0	15			
Galactose	41	159	17	44			
Glucose	5	19	2	75*			
Uronic acids	796	3330	510	803	689	25	20

* Corrected for free glucose (approximately 3 mg/5 d).

† The aldoses are analysed in one rat only.

Table 7. *Composition and faecal recovery of dietary fibre in half-soluble beet fibre*

(Mean values and standard deviations)

	Composition of dietary fibre (g/kg dry matter)	Intake (mg/5 d)		Faecal excretion					
				mg/5 d		% of intake		Corrected for basal excretion†	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Rhamnose	13	58	8	29	11	49	14	28	16
Arabinose	172	780	109	46	27	6	3	5	3
Xylose	11	49	7	28	5	57	11	47	10
Mannose	8	38	5	23	10	59	18	49	20
Galactose	41	184	26	42	15	23	7	9	6
Glucose	185	836	117	451	284*	52	29	43	30
Uronic acids	228	1030	140	182	83	17	6	17	6
Klason lignin	36	163	23	176	50	107	21		
Total	694	3140	440	976	463	30	12	26	12

* Corrected for free glucose (approximately 3 mg/5 d).

† For details, see Table 2.

Sugar-beet fibre. The half-soluble and ordinary sugar-beet-fibre preparations studied had a total dietary fibre content of 743 and 767 g/kg respectively. Of this, the soluble fraction of dietary fibre constituted 254 and 153 g/kg respectively. As shown in Tables 7 and 8, the sugar-beet fibre consists of mainly arabinose-based hemicellulose, non-starch glucans (cellulose) and pectin. The two preparations studied had similar monomeric composition.

The extent of fermentation of the various dietary fibre components was highly variable. Thus, the mean faecal recoveries (%) were: arabinose 6–12, glucose 52–58, uronic acids 17–25. The half-soluble preparation tended to be somewhat more fermented but the difference was not statistically significant. Lignin was nearly completely undegraded.

Table 8. *Composition and faecal recovery of dietary fibre in ordinary beet fibre*
(Mean values and standard deviations)

	Composition of dietary fibre (g/kg dry matter)	Faecal excretion							
		Intake mg/5 d		mg/5 d		% of intake		Corrected for basal excretion†	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Rhamnose	13	66	2	33	8	50	13	32	13
Arabinose	211	1060	27	127	87	12	8	12	8
Xylose	11	53	1	37	5	70	9	60	9
Mannose	8	40	1	22	9	55	22	44	22
Galactose	42	204	5	55	22	27	11	14	10
Glucose	191	933	24	538	182*	58	19	50	19
Uronic acids	207	1010	30	247	116	25	11	24	11
Klason lignin	62	303	8	254	93	84	30		
Total	745	3670	90	1310	340	36	9	32	9

* Corrected for free glucose (approximately 3 mg/5 d).

† For details, see Table 2.

Table 9. *Effect of various dietary fibre preparations on protein utilization by rats*
(Mean values and standard deviations)

Dietary fibre source	Additional N from dietary fibre preparations (mg/5 d)		Faecal N (mg/5 d)		True digestibility		Apparent digestibility		Biological value	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Basal diet	—	—	52.4	21.5	1.018	0.026	0.928	0.026	0.829	0.044
Wheat bran	114.4	0.5	98.4	21.9**	0.962	0.028*	0.874	0.026*	0.771	0.051
Guar gum	8.3	0.8	134.2	38.4***	0.872	0.051***	0.783	0.052***	0.843	0.039
Low-methoxyl† pectin	—	—	119.4	13.7***	0.911	0.023***	0.819	0.023***	0.799	0.050
High-methoxyl‡ pectin	—	—	149.9	23.2***	0.869	0.022***	0.777	0.022***	0.816	0.031
Half-soluble sugar-beet fibre	90.6	12.7	131.4	17.8***	0.910	0.033***	0.830	0.033***	0.744	0.043*
Ordinary sugar-beet fibre	97.9	2.5	131.0	16.2***	0.925	0.019***	0.846	0.019***	0.752	0.033*

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$.

† Low-methoxyl pectin (extent of methoxylation 37%).

‡ High-methoxyl pectin (extent of methoxylation 74%).

Protein utilization

Faecal N excretion increased when the various fibre preparations were added to the basal diet (Table 9). The increase was most pronounced with high-methoxyl pectin, followed by guar gum and the two sugar-beet-fibre preparations. There was, therefore, a decrease in both apparent and true digestibility values. The biological value of the absorbed protein was significantly lowered when the sugar-beet-fibre preparation was added to the basal diet.

Wheat bran and the two sugar-beet-fibre preparations contained significant amounts of

Table 10. *Effect of various dietary fibre preparations on fibre intake and faecal output of rats*

(Mean values and standard deviations)

Dietary fibre source	Dietary fibre intake* (g/5 d)				Faecal wet wt (g/5 d)				Faecal dry wt (g/5 d)			
	Indigestible starch		Added fibre		Mean		SD		Total		Mean increment	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Dietary fibre residue	Protein increment
Basal diet	0.25	0.03	0		2.1	0.7	1.6	0.4	—	—	—	—
Wheat bran	0.22	0.00	2.43	0.01	5.3	0.6	3.6	0.4	2.0		1.5	0.3
Guar gum	0.18	0.02	3.58	0.32	3.9	1.0	2.7	0.6	1.1		0.1	0.5
Low-methoxyl† pectin	0.19	0.02	3.37	0.43	7.0	4.3	3.4	0.9	1.8		0.7	0.4
High-methoxyl‡ pectin	0.19	0.02	3.33	0.51	7.3	3.1	4.2	1.5	2.6		0.8	0.6
Half-soluble sugar-beet fibre	0.22	0.01	3.14	0.44	5.8	2.7	3.3	0.8	1.7		1.0	0.5
Ordinary sugar- beet fibre	0.20	0.03	3.67	0.09	8.4	2.6	3.8	0.6	2.2		1.3	0.5

* The values denote dietary fibre from the different preparations added to the basal diet. The maize starch contained 8 g indigestible starch/kg which is also by definition dietary fibre.

† Low-methoxyl pectin (extent of methoxylation 37%).

‡ High-methoxyl pectin (extent of methoxylation 74%).

protein (Table 9). Thus, the increased faecal N and decreased true and apparent digestibility when feeding these types of fibre could be due to poor digestibility of the fibre-associated protein. The pectin and guar gum preparations, however, that caused the most prominent increase of faecal N, contained very little or no N. In these instances, therefore, the increase must have been due either to altered metabolic N loss or to interference with the digestion of dietary protein (casein). The most probable alternative is that the increased faecal N after feeding dietary fibre-containing diets represents bacterial protein synthesized from simple N sources, such as urea or ammonia. Whereas energy substrates are regarded as rate limiting for colonic bacterial growth (Mason & Palmer, 1973), such N sources are readily available through diffusion across the colonic wall. The lower biological value for diets containing wheat bran or sugar-beet fibre is most probably due to the additional contribution of dietary protein from these dietary fibre preparations. This can be expected to have rather low nutritional value thus decreasing the biological value of the whole diet. However, an inhibition of protein digestion by dietary fibre cannot be excluded from these experiments.

Faecal bulking

Addition of any one of the dietary fibre preparations caused a significant increase in faecal wet as well as dry weight. In the instance of bran, almost all the increased faecal loss could be accounted for by dietary fibre residues and protein (Table 10). Guar gum, on the other hand, that was almost completely fermented also gave an increased faecal mass that was only partially accounted for by the increased faecal protein. The two pectins also gave a more prominent increase in faecal dry weight than expected from faecal pectin residues and protein. It is well documented that both guar gum and pectin increase faecal fat, which probably accounts for the rest of the faecal dry weight increment after feeding these types of fibre.

GENERAL DISCUSSION AND CONCLUSIONS

The present study confirms the great variability in the extent of fermentative breakdown of various kinds of dietary fibre in the intestinal tract. The results are similar to those reported so far from human investigations. Comparative studies, in which the same dietary fibre preparations are tested both in rat and human balance experiments are needed, however, to document the usefulness of this rat experimental model to predict the physiologically-important property of bacterial fermentability exhibited by dietary fibre. Rat experiments could then be used, for instance, to study the effect of processing of fibrous foods in this respect.

The reason for the relative resistance of bran fibre to bacterial degradation is obscure. A simple physical hindrance of bacterial enzyme access to the inner core of bran particles seems unlikely in view of the fact that the bran in our diets was milled to very small particles. Furthermore, Bertrand *et al.* (1981) showed that chemical delignification did not alter the faecal recovery of dietary fibre from bran.

Extrusion cooking is increasingly used for processing cereal foods. In that process the material undergoes a prominent expansion due to heating at very high pressure and sudden introduction to atmospheric pressure at the end of the process. In a recent study (I. Björck, M. Nyman and N-G. Asp, unpublished results) we found similar proportions of dietary fibre constituents remaining in the faeces when feeding extruded and raw whole-grain wheat flour to rats. It therefore seems more likely that certain chemical bindings in dietary fibre polymers, or between such polymers, and for instance structural proteins, limit the bacterial degradation of certain kinds of dietary fibre. The structural difference between dietary fibre that is fermented and that which is not, needs further investigation.

The sugar-beet-fibre preparations studied contained similar proportions of arabinose-based hemicellulose, pectin and non-starch glucan (cellulose). Interestingly, these different components showed a completely different extent of fermentation. The arabinan was fermented almost quantitatively like the guar gum, the pectin was fermented to approximately the same extent as the purified pectins, and the glucan showed an extent of fermentation very similar to that of bran fibre. This finding provided further evidence that the chemical structure rather than the physical appearance determines the resistance to bacterial breakdown.

Since faecal polysaccharides were hydrolyzed without any previous fractionation it cannot be determined absolutely from our results whether the monosaccharides detected represented dietary fibre residues, bacterial cell-wall polysaccharides, or secreted gluco-proteins or glucosaminoglucans. The low monosaccharide levels detected on a practically fibre-free diet, and the fact that feeding easily-fermented dietary fibre like guar gum or pectin did not cause the appearance of faecal sugars other than those present in the fibre, strongly suggest that by far the major part of the faecal sugars were constituents of dietary fibre resistant to bacterial degradation. Studies are in progress to elucidate this point further.

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Dietary fibre fermentation in the rat intestinal tract
Effect of adaptation period, protein and fibre levels, and
particle size

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ABSTRACT

1. The fermentative breakdown of one resistant type of dietary fibre (wheat bran) and one easily fermented fibre (low-methoxyl pectin) was studied with respect to the length of the adaptation period and fibre level in the diet. The breakdown of the resistant fibre was also studied regarding the protein level in the diet and particle size of the fibre.
2. Prolongation of the adaptation period from 4d to 18d decreased the faecal dry weight considerably. The excretion of dietary fibre, however was similar, whereas a decrease in faecal N-excretion could be seen.
3. A level of dietary protein of less than 50 g/kg impaired the fermentation of wheat bran fibre, whereas a higher level than 100 g/kg protein did not further increase the degree of fermentation of the fibre.
4. The particle size did not change the fermentability of the fibre, equal amounts of the main components of coarse and milled bran being excreted in faeces.
5. Two different levels of wheat bran fibre (48 and 96 g/kg) in the diet did not influence the fibre breakdown. Similar results were obtained with two levels of fibre from pectin (42 and 84 g/kg), but a tendency towards a decreased percent faecal excretion of uronic acids was seen at the lower pectin fibre level.

INTRODUCTION

The extent of dietary fibre fermentation in the intestinal tract varies between different types of fibre, and is dependent upon the solubility and the chemical structure of the fibre [Cummings, 1982]. Dietary fibre rich in lignin and cellulose, such as wheat bran, is highly resistant to micro-organisms in the intestinal tract, whereas hemicellulosic and pectic substances ferment more complete [Cummings et al. 1979; Dintzis et al. 1979; Gramstorff Fetzner et al. 1979; Bertrand et al. 1981; Nyman & Asp, 1982]. Fermentation is the main factor determining the bulking capacity of dietary fibre. The low bulking capacity of easily fermented fibre is due to increased bacterial mass [Stephen & Cummings, 1980]. In contrast, the increase in faecal dry matter by wheat bran is mainly due to unfermented fibre. The capacity of the unfermented fibre to bind water is also of importance for the faecal bulking capacity. Dietary fibre with a high water-binding capacity in vitro have a poor bulking capacity, however, due to the extensive fermentation of this kind of fibre [Stephen & Cummings, 1979].

The fermentability of a certain dietary fibre can also be expected to be influenced by a number of other factors such as particle size of the fibre, and also by different non-fibre components in the diet, e.g. the protein content. Heller et al. (1980) found that the fermentation of cellulose was more pronounced with fine bran, than with coarse bran in man, while Ehle et al. (1982) showed that coarse bran was more susceptible to the microflora in pigs. The importance of particle size has been further established by Brodribb & Groves (1978), who found a significantly higher wet weight of faeces in man with coarse bran than with finely ground bran.

Adaptation of the intestinal microflora is another factor that might affect fibre degradation, especially for an easily fermented type of fibre. In a previous investigation (Nyman & Asp, 1982) it was shown that the range between rats was considerable with pectins, which could be due to the rats being in different phases of adaptation. One could therefore expect that a longer adaptation period to the diet, would decrease this variation. Cunningham et al. (1962), however, did not find any increase in fermentability when pigs were given a resistant dietary fibre (Solka - Floc) for a longer time (14 weeks compared with 1 week), and Cummings et al. (1982) found the same resistance of wheat bran fibre after six weeks compared to three weeks in man. Further, Ehle (1980) did not observe any long term adaptation of the faecal microflora to the fibre in humans and pigs. The level of fibre in the diet can also be expected to affect fibre breakdown. Keys et al. (1970) demonstrated that fibre digestibility decreased with increasing fibre level.

The present study was undertaken to provide information on whether parameters such as adaptation time to the diet, level of protein or fibre in the diet, and particle size could change the fermentability of the fibre. A resistant dietary fibre (wheat bran) and an easily fermented fibre (low-methoxyl pectin) were investigated in balance experiments with rats. This rat model appears to be useful in predicting dietary fibre breakdown in man.

MATERIALS AND METHODS

Dietary fibre preparations

Two batches of wheat bran (Kungsörnen, Sweden and Dunn Clinical Nutrition Unit, Cambridge, England), one batch of enzyme-treated, concentrated and dephytinized wheat bran, referred to as processed bran (Fiberform, Tricum AB, Höganäs, Sweden), and one low-methoxyl pectin (Copenhagen Pectin Factory Ltd., Denmark) were used. The dietary fibre preparations were milled to a particle size less than 0.4 mm, except when the effect of particle size was studied.

Diets

The diets contained in g/kg: dietary fibre 42-100, casein as the source of protein 0-200, sucrose 100, maize oil 50, mineral mixture 48 and vitamin mixture including choline chloride 10 (for details regarding vitamin and mineral mixtures see Nyman & Asp, 1982). Maize starch was added to adjust dry matter content. A basal diet with no added fibre and with 100 g/kg casein was also included as a control. The diets were mixed without heat treatment and fed as powders.

Protein level. Casein 0, 50, 100, 150 and 200 g/kg was used when investigating effects of the protein level. Wheat bran 49 g fibre/kg, was used as source of fibre in these experiments. When evaluating the effects of adaptation time, fibre level and particle size, a level of protein of 100 g/kg was used.

Adaptation time. 100 g processed wheat bran fibre/kg or 84 g pectin fibre/kg was used and given to rats for a short (4d) and a long (18d) adaptation period, respectively.

Dietary fibre level. Dietary fibre was added in the following concentrations (g/kg): Wheat bran 48 or 96 and low-methoxyl pectin 42 or 84.

Particle_size. Milled and coarse (particle size 0.4-1.4 mm) processed wheat bran fibre was investigated.

Balance experiments

Male Sprague Dawley rats, 80 g, were divided into groups of five and placed individually in metabolic cages (Nyman & Asp, 1982). The food intake was restricted to 10 g dry weight/d. Water was provided ad_lib. After a 4 d adaptation period, feed residues and faeces were collected during a 5 d balance period. The weight of the rats at the beginning of the balance period was about 90 g. Faeces were removed every day and frozen at -20° . The faeces were lyophilized, weighed, milled and stored at -20° until analysis.

A longer adaptation period, 18 d, was also tested for processed wheat bran and low-methoxyl pectin. The experimental diet was then provided ad_lib to rats weighing 40 g, during the first fourteen days. Groups with short adaptation periods were fed standard pellets during the same time. The experimental diet (10 g dry weight/d) was then given for another 4 d to the different groups, followed by a 5 d balance period, when feed residues and faeces were collected. The weight of these rats at the beginning of the balance period was about 100 g.

Analyses

Fibre analyses. Dietary fibre content was measured gravimetrically after digestion with physiological enzymes using the method of Asp et al. (1983). Dietary fibre residues were corrected for protein ($N \times 6.25$) and ash associated with the fibre.

Dietary fibre composition in feed and in faeces was measured with gas-liquid chromatography (GLC) of neutral sugars as their alditol acetates (Sawardeker et al. 1965; Theander & Aman, 1979). Allose was used as internal standard. The hydrolysis was performed in 12 M H_2SO_4 for 2 h and then diluted and reflux boiled for 6 h. Uronic acids were measured with a decarboxylation method (Bylund & Donetzhuber, 1968; Theander & Aman, 1979). All dietary fibre constituents were expressed as their polymer weight.

N-determination. N in food, urine and faeces was determined by using the Kjeldahl method. Crude protein was calculated as $N \times 6.25$.

Statistical evaluation. The materials in Table 1 were analysed by Dunnett's method for comparing several groups with one control-group (Winer, 1971). Table 4 was analysed by a one way analysis of variance design followed by the Newman-Keul's procedure for multiple comparisons (Winer, 1971). Other statistical evaluations were made by Student's t-test.

Calculation. All dietary fibre constituents excreted in faeces, were corrected for the basal excretion, i.e. typical "dietary fibre" saccharides excreted in faeces, when the rats were fed a basal diet with no added fibre (Nyman & Asp, 1982). The sum of basal excretion of "dietary fibre" monomers was about 120 mg/5 d. The glucan values were also corrected for the free glucose found in faeces (less than 10 mg/5 d).

RESULTS AND DISCUSSION

Faecal dry weights

The faecal dry weights obtained with milled and coarse processed wheat bran were similar (Table 1). On the other hand, faecal dry weight with processed wheat bran after a long adaptation period, was significantly ($p < 0.01$) lower than that after a short one. The faecal dietary fibre excretion, however, was similar after long and short adaptation periods, whereas protein ($p < 0.001$), and probably also fat excretion was lower after the long adaptation period. Thus the longer adaptation leads to less faecal protein and fat losses, whereas dietary fibre fermentation was unaffected. A prolonged adaptation with low-methoxyl pectin (84 g/kg) also caused a lower mean faecal dry weight. However, since there was a considerable variation between rats, especially when the short adaptation period was used, the decrease was not significant. The decrease in faecal dry weight, with the longer adaptation period (0.8 g/5d) seemed to be due to a decreased excretion of dietary fibre (not significant), protein ($p < 0.01$) and probably fat.

Thus, a prolonged adaptation to the fibre containing diet, decreased faecal dry weights, due to a decreased excretion of protein and probably fat, whereas the excretion of fibre was practically unchanged. The particle size had no pronounced effects on faecal dry weights. Any conclusions on faecal wet weights in relation to particle size cannot be drawn since the rat faeces dry between collections.

Other studies have shown an importance of particle size, for faecal wet weights (Heller et al. 1980; Brodribb & Groves, 1978, Van Dokkum et al. 1983). Results on the effect of particle size on faecal dry weights are more ambiguous (Heller et al. 1980; Ehle et al. 1982; Van Dokkum et al. 1983). However, according to Heller et al. (1980) faecal dry weights

were significantly higher in humans given a coarse bran diet than in humans given a fine bran diet.

Fermentation of dietary fibre components

In the present study faecal polysaccharides were determined without any previous fractionation and it can be questioned whether the saccharides detected represents dietary fibre residues, bacterial cell-wall polysaccharides or endogenous polysaccharides. However, there is evidence that most of the saccharides excreted in faeces originate from the fibre. When rats were given a starch based diet, with no added fibre, a very low level of saccharides was detected in faeces (Nyman & Asp, 1982). Of course it can not be excluded that the excretion will increase with fibre in the diet. Guar gum, however, which may stimulate the microbial activity in the large intestine, through its considerable fermentability did not significantly increase the excretion of faecal saccharides compared to a fibre-free diet (Nyman & Asp, 1982). In addition no other saccharides than those present in the fibre could be detected in considerable amounts in faeces (Nyman & Asp, 1982) and the pentoses arabinose and xylose, which are the main components in wheat bran, are very rare in bacterial saccharides (Kenne & Lindberg, 1983). Thus by far most of the saccharides excreted in faeces are fibre saccharides.

Adaptation period: The faecal recovery of the main dietary fibre constituents with processed wheat bran was very similar after both adaptation periods (Table 2). The mean excretion in faeces was 69 and 68% for arabinose, 46 and 44% for xylose and 65 and 69% for glucose, with the short and long adaptation periods, respectively. In the case of low-methoxyl pectin there was a small decrease in the mean excretion of uronic acids with a longer adaptation period, but no significant

differences were detected (Table 3). 27% were excreted when a short adaptation period was used compared with 21% with a prolonged period.

Thus the adaptation period had no pronounced effects on the extent of fermentation on a resistant type of dietary fibre, or on an easily fermented one. However, the variation between different rats with an easily fermented type of fibre seemed to decrease with a longer adaptation period.

Similar fermentability of the fibre after different lengths of the feeding time, have also been reported by other authors. Cunningham et al. (1962) found the same cellulose digestibility (Solka-Floc) in pigs after 1 and 14 weeks. Furthermore, no long term adaptation of the faecal microflora to the fibre has been observed in man or pigs (Ehle, 1980).

Protein level: The fermentation of the total fibre was most extensive at a protein level of 100 g/kg, followed by 200, 50, 150 and 0 g/kg, respectively (Table 4). The main dietary fibre constituents showed a similar trend. One way analysis followed by multiple comparisons (Newman-Keul's test) showed that the excretion of total fibre at protein levels of 100 and 200 g/kg was significantly ($p < 0.01$) lower compared to a protein level of 0 g/kg. No significant differences between arabinose values were obtained, or between glucose values. The faecal excretion of xylose after the diet with no added protein was significantly ($p < 0.01$) higher than after the other diets. Thus, protein (nitrogen) seemed to be a limiting factor for dietary fibre fermentation at dietary protein levels lower than 50 g/kg. However, a level of protein higher than 100 g/kg that is used routinely, did not increase the fermentability of the fibre further (Nyman & Asp, 1982; Björck et al. 1984).

Fibre level: The extent of fermentation of wheat bran fibre was similar when given at two different levels (48 and 96

g/kg) (Table 5). In both experiments 61% of the total fibre was recovered in faeces. Of the arabinose 63% was excreted in faeces and of the xylose 48%. The percent excretion of glucose-based fibre, however, was reduced ($p < 0.05$) when the level of ingested fibre decreased.

The fermentability of low-methoxyl pectin did not change significantly when the level of fibre was reduced (Table 3). However, there was a tendency towards a decreased faecal excretion of uronic acids as well as a decreased variation between rats at the lower level.

Thus, the fermentability of both resistant and easily fermented types of fibre seems to be independent of the fibre level given, at least at the concentrations used in this study. This suggests that certain chemical bonds in the polysaccharides are resistant to bacterial fermentation. This is further supported by the fact that the adaptation time seems to be rather unimportant.

The results obtained in the present study are in agreement with other studies (Kies et al. 1984; Nyman et al. 1985), reporting similar resistance of cellulose given at different levels. More over, Garrison et al. (1978) could not detect any significant effects on the fibre breakdown in rats given increasing levels of fibre from bagasse. In contrast, Frank et al. (1983) found a decreased digestibility of corn cob fibre in pigs with increasing dietary fibre levels. Similar results were obtained by Keys et al. (1970). However, since quite different fibre levels were tested in the different investigations, it is difficult to compare them. Another complicating factor is the different methodologies used when measuring dietary fibre.

Particle size. Very similar amounts of the main components in coarse and milled processed bran were excreted in faeces (Table 2). The faecal recovery with milled and coarse bran,

was 69 and 70% for arabinose, 46 and 43% for the xylose and 65 and 70% for glucose, respectively.

It is generally assumed that the particle size per se does affect the susceptibility to bacterial degradation of the fibre [Cummings, 1982], but earlier studies are not consistent. Heller et al. (1980) found a decrease (not significant) in the susceptibility to bacterial fermentation in man given coarse bran compared to fine bran, whereas Ehle et al. (1982) reported a slight increase of the fermentation of cellulose in pigs given coarse bran. They interpreted this as an effect of the increased transit time. Our results are in agreement with Björck et al. (1984) who found the same dietary fibre content in faeces from rats fed extruded, i.e. a process that increase the surface area of particles, and unprocessed whole-grain wheat flour.

CONCLUSIONS

In conclusion, a 4 d adaptation to the diet and a protein level of 100 g/kg seem adequate when evaluating the fermentability of dietary fibre in rats. These kinds of balance experiments have also been shown to correlate well with balance experiments in man (M.Nyman, N.G.Asp, J.Cummings & H.Wiggins, unpublished results) where apple, cabbage, carrot, guar gum and bran were found to be fermented to similar extents in man and in rats.

The particle size per se did not affect faecal dry weight or the fermentability of the fibre. Neither was the fermentation affected by the level of fibre in the diet.

Prolongation of the adaptation period decreased faecal dry weight, due to a decreased excretion of protein and probably also fat, but the fermentation of the fibre was practically unchanged.

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Table 1
Dietary fibre intake and faecal dry weights
(Mean values and standard deviations)

	Dietary fibre intake (g/5d)		Faecal dry wt (g/5 d)		Fibre residue ^a	Protein increment
	Mean	SD	Total			
			Mean	SD		
Basal diet						
Low-methoxyl pectin						
LS-42 ^b	2.1	0.0	1.4	0.4	-	0.4
LS-84 ^b	4.0	0.2	2.5	0.5**	1.1	0.5
LL-84 ^b	4.0	0.3	3.4	1.1***	2.0	1.3
Wheat bran						
W-48 ^c	2.1	0.0	2.6	0.5**	1.2	1.0
W-96 ^c	4.4	0.3	3.2	0.3***	1.8	1.5
W+0 ^d	1.6	0.2	5.1	0.4***	3.7	0.4
W+50 ^d	2.0	0.4	3.0	0.2***	1.6	0.0
W+100 ^d	2.5	0.0	3.6	0.9***	2.2	0.3
W+150 ^d	2.4	0.1	3.6	0.4***	2.2	0.3
W+200 ^d	2.4	0.0	3.9	0.6***	2.5	0.5
Fiberform						
WS ^e	5.0	0.0	3.5	0.5***	2.1	0.5
WL ^e	5.0	0.0	6.2	0.3***	4.8	0.5
WS-C ^e	5.0	0.1	5.3	0.3***	3.9	0.3
			6.4	0.5***	5.0	0.6

***p<0.001 **p<0.01 *p<0.05 compared with the basal diet group (Dunnett's method).

^aIncluding basal excretion of "dietary fibre" (approximately 120 mg/5 d) and free glucose (approximately 10 mg/5 d).

^bFirst letter; low-methoxyl pectin (L), Second letter; short (S) and long (L) adaption period. Figures; the level of dietary fibre in the diet (g/kg).

^cFirst letter; wheat bran (W), Figures; the level of dietary fibre in the diet (g/kg).

^dFirst letter; wheat bran -49 g fibre/kg (W), Figures; the level of protein in the diet (g/kg).

^eFirst letter; processed wheat bran -100 g fibre/kg (W), Second letter; short (S) and long (L) adaptation period, Third letter; coarse (C) particles.

Table 2 Composition and faecal recovery of dietary fibre with the processed wheat bran
(Pooled samples from five rats)

	Composition of dietary fibre (g/kg dry matter)	Faecal excretion					
		mg/5 d		% of intake			
		Short adaptation	Long adaptation	Short adaptation- coarse	Short adaptation	Long adaptation	Short adaptation- coarse
Rhamnose	-	6	10	7			
Arabinose	158	660	630	675	69	68	70
Xylose	251	710	680	660	46	44	43
Mannose	7	47	50	44	109	105	105
Galactose	13	63	94	55	80	84	70
Glucose	198	790	840	840	65	69	70
Uronic acids	26	160	190	110	100	119	69
Lignin	157	1080	970	1140	112	101	118
Total	810	3520	3460	3530	71	69	71

Table 3 Composition and faecal recovery of dietary fibre in low-methoxyl pectin
(Mean values and standard deviations)

Composition of dietary fibre (g/kg dry matter)	mg/5 d		Faecal excretion % of intake			
	Short	Short	Long	Short	Short	Long
	adaptation- 42g fibre/kg Mean SD	adaptation- 84g fibre/kg Mean SD	adaptation- 84g fibre/kg Mean SD	adaptation- 42g fibre/kg Mean SD	adaptation- 84g fibre/kg Mean SD	adaptation- 84g fibre/kg Mean SD
Rhamnose 7	2 ^a	17 ^a	0 ^a			
Arabinose 2	42 ^a	13 ^a	15 ^a			
Xylose 1	9 ^a	6 ^a	7 ^a			
Mannose 1	8 ^a	7 ^a	5 ^a			
Galactose 3	7 ^a	31 ^a	16 ^a			
Glucose 4	16 ^a	73 ^a	40 ^a			
Uronic acids 873	320 170	1020 650	750 430	22 1 ^{ns}	27 18	21 1 ^{ns}

*** p<0.001 ** p<0.01 * p<0.05 ns=not significant (Student's t-test) compared with the reference group i.e. short adaptation -84 g fibre/kg.

^a Pooled samples from five rats

Table 4 Faecal recovery of dietary fibre in wheat bran with different levels of protein in the diet - 0, 50, 100, 150, and 200 g/kg (Mean values and standard deviations)

Composition of dietary fibre (g/kg dry matter)	Faecal excretion												% of intake ^a							
	mg/5 d																			
	0g/kg		50g/kg		100g/kg		150g/kg		200g/kg		0g/kg		50g/kg		100g/kg		150g/kg		200g/kg	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Rhamnose	5	4	4	4	5	4	10	5	1	2										
Arabinose	210	29	240	61	270	22	290	35	270	31	65	8	59	7	57	5	60	6	55	6
Xylose	340	37	300	74	320	17	380	65	320	76	73	5	50	3	45	2	54	8	45-11	
Mannose	7	2	7	4	9	1	17	6	9	3	62	14	49	21	50	7	55	59	48	18
Galactose	3	4	19	11	15	6	19	8	7	5	9	9	46	26	29	13	38	15	19	10
Glucose	270	40	310	100	330	30	380	37	340	41	64	7	56	8	51	5	59	4	53	6
Uronic acids	5 ^b		1 ^b		10 ^b		5 ^b		6 ^b		29 ^b		6 ^b		56 ^b		20 ^b		24 ^b	
Total	840	99	880	240	960	48	1100	130	950	150	65	5	54	5	49	2	57	6	49	8

^aStatistical analyses of the material were performed with one-way analysis of variance followed by the Newman-Keul's method (See text).

^bpooled samples from five rats.

Table 5 Faecal recovery of dietary fibre in wheat bran, with two different levels of dietary fibre, (48 g/kg and 96 g/kg)
[Mean values and standard deviations]

	Composition of dietary fibre (g/kg dry matter)	Faecal excretion % of intake					
		mg/5 d					
		48g/kg	96g/kg	48g/kg	96g/kg	48g/kg	96g/kg
		Mean	SD	Mean	SD	Mean	SD
Rhamnose	-	15	6	15	3	-	-
Arabinose	133	290	32	620	28	63	6
Xylose	183	300	18	640	45	47	2
Mannose	3	17	4	27	7	140	35
Galactose	11	28	3	45	5	5	4
Glucose	169	420	34	910	44	60	7*
Uronic acids	31	80 ^a		80 ^a		47 ^a	33 ^a
Klason lignin	67	250	28	470	73	110	12
Total	597	1400	30	2810	140	61	2
						61	3

*** $p < 0.001$ ** $p < 0.01$ * $p < 0.05$ (Student's t-test).

^apooled samples from five rats.

Fermentation of dietary fibre in the intestinal tract
Comparison between man and rat

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ABSTRACT

1. The breakdown and faecal bulking capacity of dietary fibre preparations from wheat bran, apple, cabbage, carrot, and guar gum were compared in man and rat.
2. The degradation of the fibre showed good correlation between man and rat ($r = 0.97$). Wheat bran, was the least well-digested, 66 and 59 % of the neutral sugars being excreted in faeces of man and rat, respectively. The breakdown of the fibre in apple, cabbage, carrot and guar gum was more complete and 4-30% of the neutral sugars were recovered in faeces.
3. The main dietary fibre constituents in each preparation were degraded to similar extent in man and rat. The main dietary fibre constituents of apple, carrot, cabbage and guar gum were almost completely degraded. Of the xylose in wheat bran 45% (man) and 48% (rat) were recovered in faeces. However, the percent excretion of glucose and arabinose from bran was higher in man.
4. A faecal glucan other than cellulose was identified in the human faeces after guar gum, and has been provisionally identified as starch. No such glucan occurred in the rat faeces.
5. A good correlation between the faecal bulking capacity in man and rat was seen ($r=0.95$). Wheat bran had the best bulking capacity, while that of apple, cabbage, carrot and guar gum was less pronounced. Faecal bulking was inversely related to the amount of fibre which was water-soluble in each preparation.
6. It is concluded that this rat experimental model is useful for prediction of fermentative breakdown and bulking capacity of dietary fibre in man.

INTRODUCTION

The extent of fermentative breakdown of dietary fibre in man, is important in relation to its physiological effects (Royal College of Physicians, 1980; Cummings, 1981). Dietary fibre, such as that from wheat bran, which is largely resistant to bacterial degradation has good bulking capacity in the gut while easily-fermented dietary fibre, such as that found in non-cereal foods, can be utilized as a source of energy by bacteria (Mason & Palmer, 1973) and also by man (Mc Neil et al. 1978). Fermentation increases colonic bacterial activity, and leads to changes in bile acid and nitrogen metabolism (Cummings 1983a) and the synthesis of vitamins (Rotenberg et al. 1982). The possible protective effect of dietary fibre against large bowel cancer may be related to the metabolic activity of the intestinal flora (Cummings & Branch, 1982).

The extent of fermentation of fibre is influenced by a number of factors, such as water-solubility, chemical structure of the fibre (Cummings, 1982; Nyman & Asp, 1982), lignification (Chandler et al. 1980), particle size (Heller et al. 1980), other components of the diet and the amount ingested (Keys et al. 1970). Processing of dietary fibre may also affect its degradation. Björck et al. (1984) demonstrated that dietary fibre of extruded wheat flour was more extensively degraded in rats than dietary fibre of unprocessed wheat flour, and Wyman et al. (1976) suggested that the faecal bulking effect of raw bran in man was more pronounced than that of cooked bran.

Balance studies on humans are complicated and time consuming. Consequently there is a need for a simpler model to predict the degree of dietary fibre fermentation in human nutrition. However there are differences between man and rat e.g. bigger caecum in rat, that may be important for the bacterial degradation of dietary fibre. Van Soest et al. (1983) have done comparative studies on the fermentation of dietary fibre in man and other animals, and concluded that that in man it was more extensive than in rats but less than in swine.

This study reports a detailed comparison between man and rat of intestinal degradation of dietary fibre from bran, apple, cabbage, carrot and guar gum.

MATERIALS AND METHODS

Dietary fibre preparations

Dried carrot, cabbage, bran and apple were extracted twice for 3h with hot 85% v/v aqueous methanol, then, after filtration, with acetone and finally air dried. The materials were ground, sieved and the particles retained between 0.5 and 1.4 mm sieves used for the feeding studies. Guar gum was obtained from the Hercules powder Co. Further details of the methods of preparation have been reported previously (Cummings et al. 1978).

Human experiments

Diets. The subjects ate a controlled basal diet, throughout the study, which contained normal food and was of the following composition (g/kg dry matter): Protein 142, carbohydrates 642, fat 180, dietary fibre 37. During one dietary period 20-30 g of the fibre preparations, were added to the basal diet. Further details of these studies have been presented elsewhere (Cummings et al. 1978).

Experimental. Nineteen healthy male volunteers aged 20-38 took part in the study. Each subject took the controlled basal diet for three weeks. Faeces were collected throughout the 3-week period and for 1 week afterwards, each stool being collected separately into a plastic bag and stored at -20° . In another 3 week period additional fibre was added to the basal diet. At least 3 weeks elapsed between the two dietary periods to ensure that one dietary period had no residual effect on the next one. All the subjects took 10 radio-opaque pellets with each meal (30/d) throughout the study as markers for faecal output. Six men took fibre from more than one source (Cummings et al. 1978).

Fibre analyses. Fibre in the diet was obtained by calculations from food tables (Paul & Southgate, 1978). Faecal carbohydrates were measured by gas-liquid chromatography (GLC) after hydrolysis of non-cellulosic polysaccharides with 1M sulphuric acid. Cellulose was measured by a colorimetric assay of the glucose released when the insoluble residue from the 1M sulphuric acid hydrolysis was incubated with 12M H_2SO_4 (Englyst et al. 1982).

Rat experiments

Diets. The basal diet contained (g/kg dry matter): protein (in the form of casein) 100, maize starch 690, sucrose 100, fat 50, vitamins including choline chloride 10, and minerals 50. The dietary fibre preparations were substituted for maize starch to give 100 g dietary fibre/kg dry matter (Nyman & Asp, 1982).

Animals. Male Sprague Dawley rats, three weeks old, with an initial weight of 75 g, were divided into groups of five. Food intake was restricted to 10 g dry weight/d. Water was provided ad lib. A 4 d adaptation period was followed by a 5 d balance period, when feed residues and faeces were collected, stored at -20° and lyophilized (Nyman & Asp, 1982).

Fibre analyses. Dietary fibre in the various preparations was isolated by the gravimetric method of Asp et al. (1983), using digestion with Termamyl (120L, Novo, Copenhagen) pepsin (No. 7190, Merck, Darmstadt) and pancreatin (No P-1750, Sigma, St. Louis, USA). The monomeric composition of the neutral sugars in the fibre residues and in faeces was assayed by GLC as the alditol acetates (Sawardeker et al. 1965). Hydrolysis was started in 72% H_2SO_4 (5ml/100 mg) at room temperature for 2 h. The sulphuric acid was then diluted to 0.36 M and refluxed for 6 h (Theander & Åman, 1979). The insoluble residue was measured as Klason lignin. Allose was used as the internal standard in the gas-liquid chromatographic assays. The amount of uronic acids in feed and in faeces was measured with a decarboxylation method (Bylund & Donetzhuber, 1968 ; Theander & Åman, 1979). All dietary fibre constituents were expressed as their polymers, i.e. weight of monomer x 0.9. In addition, a correction for hydrolytic losses, detector response and recovery in the GLC method was made by running the GLC procedure with known sugar standards.

The sum of neutral sugar polymers, uronic acid polymers and Klason lignin amounted to 96-103 % of the total dietary fibre measured gravimetrically according to Asp et al. (1983).

Calculation

Faecal excretion of dietary fibre components, in the human and rat experiments, was corrected for basal excretion, i.e. the faecal excretion of dietary fibre constituents on the basal diet. In the rat experiments the glucans excreted in faeces were also corrected for the free glucose present.

RESULTS AND DISCUSSION

Fermentation of neutral sugars

Wheat bran. The wheat bran preparation contained 597g/kg dietary fibre consisting mainly of arabinose, xylose, glucose and lignin in expected proportions (Table 1).

The fermentative breakdown of the dietary fibre saccharides averaged 34% in man and 41% in rat (Table 1 and Fig. 1). The faecal recovery of the main components, arabinose, xylose and glucose were: 73, 45 and 93% in man and 63, 48 and 68% in rat, respectively. Thus, xylose values were similar in man and rat, whereas faecal excretion of arabinose and glucose was higher ($p < 0.05$) in man.

Apple. The apple-fibre preparation studied contained 766g/kg dietary fibre, with 50% glucose and 20% uronic acids. Significant amounts of arabinose, xylose, galactose and lignin were also detected (Table 2).

The fermentability of the fibre in apple was very similar in both man and rat. The faecal recovery of the neutral sugars averaged 19% in man and 22% in rat (Table 2 and Fig. 1). Excretion of the main components arabinose, xylose, galactose and glucose was also similar in the two species. Although, there was considerable individual variation in both man and rat fed with this material the mean values were in good agreement.

Cabbage. The dietary fibre content in the cabbage preparation was 703g/kg. Approximately 70% of the fibre consisted of glucose and uronic acids. Other fibre components detected in significant amounts were arabinose and galactose (Table 3).

Cabbage was an easily-fermented type of dietary fibre and only 10 and 13% of the neutral fibre saccharides were recovered in faeces from man and rat, respectively (Table 3 and Fig. 1). Both species showed similar ability to degrade the main components, arabinose, galactose and glucose.

Carrot. The total dietary fibre content in the carrot preparation was 799g/kg. The relative distribution of main components was rather simi-

lar to that in cabbage (Table 4).

Similar amounts of the neutral fibre-saccharides were recovered in faeces in both species. The faecal recovery was 25% in man versus 29% in rats (Table 4 and Fig.1). The main neutral dietary fibre components arabinose, galactose and glucose also showed a similar availability to bacterial breakdown. However, there was a large individual variation, especially in the human experiment.

Guar gum. Guar gum, which is a rather pure galactomannan, was almost completely fermented in rat, whereas in man it seemed somewhat more resistant (Table 5). 13% of the neutral sugars was excreted in man versus 4% in rat. However, during the guar feeding period in man additional xylose, glucose and rhamnose+fucose appeared in faeces. The additional fucose and possibly some of the glucose excreted could be explained by increased mucus secretion. However, most of the glucose excretion was derived from a glucan that had many of the characteristics of starch in vitro. The large standard deviation of glucose is because that one subject excreted very large quantities of this material. In the rat experiment glucan excretion, corrected for free glucose, was 75% of the ingested amount. The glucan, however, was a minor component (<10g/kg) in the guar gum preparation, and the rat experiment did not provide evidence for faecal starch excretion during feeding with guar gum. If excluding the faecal excretion of these non-fibre saccharides and only considering the excretion of the main fibre components, mannose and galactose, the correlation between man and rat was better. Less than 2% of the mannose and the galactose being recovered in faeces of both species (Fig.1).

Fermentation of uronic acids and lignin

In the present study the faecal excretion of uronic acids and lignin was analysed in rats but not in man. However, the results obtained in rats were very much the same as those reported earlier from human investigations.

No bacterial degradation of lignin was seen in rats, which is in good agreement with studies in man (Dintzis et al. 1979; Van Dokkum et al. 1983). The uronic acids in apple, cabbage and carrot were nearly completely fermented in rats, only 7-12% of the intake could be detected

in faeces. These results are similar to those obtained in man. Pure pectin, mostly consisting of uronic acids, has been shown to be completely fermented (Cummings et al. 1979). Thus, the fermentability of uronic acids and lignin can be assumed to be similar in man and rat.

Faecal bulking capacity

A good correlation ($r=0.95$) between faecal dry weight in man and rat was seen (Fig.2). Wheat bran, as many studies have shown, produces the largest effect on stool weight, that of apple, cabbage, carrot and guar gum being much less pronounced. The faecal dry weight increment in rats on guar was due to increased excretion of fat and protein (Nyman & Asp, 1982).

The amount of soluble fibre, measured with the gravimetric method of Asp et al. (1983), was quite different in the various fibre preparations. 23-29% of the fibre in apple, cabbage and carrot was soluble versus 8% of the wheat bran fibre. In contrast, guar gum was almost completely soluble. Figure 1 and 2 show that the more soluble fibre the greater the extent of its degradation in the gut and the smaller the increment in stool weight it produces.

GENERAL DISCUSSION AND CONCLUSIONS

These studies show that breakdown of dietary fibre in the intestinal tract was similar in both man and rat. On the whole soluble dietary fibre was more readily broken down than insoluble (Fig.1). Whatever method is used to measure dietary fibre and its components it is clear that the major part of it is degraded in the gut, with the exception of wheat bran fibre.

The individual variation in the breakdown of dietary fibre, i.e. the standard deviation, was much smaller for wheat bran (Table 1) than for apple and carrot (Table 2 and 4) in both man and rat. It could be questioned if a longer adaptation period to the diet would have reduced the standard deviation. Earlier studies on rats have shown, that the individual variation in fibre breakdown tended to go down with time after feeding with an easily-fermented type of fibre (Nyman & Asp, 1985). The composition of the colon microflora however has been reported to be relatively constant in man, and cannot be fundamentally changed by nutrition (Savage, 1983). The differences between individuals are more pronounced than the alterations achieved after changes in the diet (Cummings, 1983b).

In most materials, including those studied in the present investigation the total glucan value is a good measure of the cellulose content (Theander & Åman, 1981). In the rat experiments 32-81% of this glucan was fermented and there was no indication of an increased glucan excretion due to any of the fibre preparations tested. In the human study cellulose was measured specifically and it was clear that some of the excreted glucan was not cellulose but a substance that behaved similarly to partly degraded starch. In the study of guar gum, which contains no significant glucose, large amounts of this non-cellulosic glucose were excreted particularly by one subject, who also had a fast transit time. Starch resistant to amylase is known to occur in human foods (Englyst et al. 1982; Johansson et al. 1984; Siljeström et al. 1985). It may be broken down incompletely in the large intestine either because there is insufficient time for microbial action, or in the presence of alternative carbohydrates substrates which are more soluble, such as guar gum, resistant starch is saved.

Van Soest et al. (1983) have observed in a series of comparative studies of fermentation of dietary fibre in man and other animals that it is largely dependent on the body size. Larger animals such as man and pigs digest fibre to a greater extent than smaller animals, such as the rat. They have therefore suggested, that rodents are of no use when studying problems in human nutrition. Small animals need more energy per unit of body weight, which means faster transit and lower fibre utilization.

In our study there was no indications to a more extensive degradation of fibre in man than in rats. In contrast wheat bran was somewhat more resistant to bacterial degradation in man. On the whole however the agreement was good, most fibre being totally degraded. Thus, it seems that this rat model can be used to predict bacterial degradation and the bulking effect of various types of dietary fibre in man, and to study the effects of various processes, e.g. extrusion cooking and baking on dietary fibre.

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Table 1. Composition and faecal recovery of dietary fibre in wheat bran
(Mean values and standard deviations)

	Dietary fibre composition† (g/kg dry matter)	Intake		Faecal excretion	
		Man (g/d)	Rat (g/d)	Man (%)	Rat (%)
		Mean	SD	Mean	SD
Rhamnose + Fucose	-	-	-	-	-
Arabinose	133	3.72	0.196	73	5**
Xylose	183	5.88	0.269	45	4
Mannose	3	0.11	0.005	36	29
Galactose	11	0.26	0.017	94	29
Glucose	169	3.70	0.248	93	21
Sum of neutral sugars	499	13.67	0.735	66	9*
Uronic acids	31	0.43	0.046	-	†
Klason lignin	67	2.04	0.098	-	†
Total	597	16.14	0.877	-	†

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to rats
† Isolated by using the method of Asp et al (1983) and further characterized by GLC
+ (Theander & Aman, 1979)
+ Not analysed
" Pooled samples from five rats

Table 2. Composition and faecal recovery of dietary fibre in apple
(Mean values and standard deviations)

	Dietary fibre composition* (g/kg dry matter)	Intake		Faecal excretion	
		Man (g/d)	Rat (g/d)	Man (%) Mean SD	Rat (%) Mean SD
Rhamnose + Fucose	10	0.27	0.010	27 29	50 15
Arabinose	60	1.40	0.058	5 5	4 4
Xylose	59	1.33	0.057	12 11	15 10
Mannose	22	0.13	0.021	23 29	27 18
Galactose	55	1.08	0.053	4 12	7 7
Glucose	360	7.78	0.346	26 38	27 19
Sum of neutral sugars	566	11.99	0.545	19 26	22 15
Uronic acids	159	5.73	0.153	- [†]	7 7
Klason lignin	41	0.65	0.039	- [†]	114 9
Total	766	18.37	0.737	- [†]	24 12

* Isolated by using the method of Asp et al (1983) and further characterized by GLC
+ (Theander & Aman, 1979)
Not analysed

Table 3. Composition and faecal recovery of dietary fibre in cabbage
(Mean values and standard deviations)

	Dietary fibre composition* (g/kg dry matter)	Intake		Faecal excretion	
		Man (g/d)	Rat (g/d)	Man (%)	Rat (%)
		Mean SD		Mean SD	Mean SD
Rhamnose + Fucose	16	0.41	0.020	31 11	20 7
Arabinose	101	2.97	0.129	2 3	3 1
Xylose	27	1.01	0.034	6 6	21 6
Mannose	15	0.41	0.019	8 4	14 8
Galactose	58	1.42	0.074	8 8	2 3
Glucose	220	5.68	0.280	13 10	19 10
Sum of neutral sugars	437	11.90	0.556	10 7	13 6
Uronic acids	252	6.33	0.321	- [†]	12 9
Klason lignin	14	0.18	0.018	- [†]	124 53
Total	703	18.40	0.895	- [†]	15 8

* Isolated by using the method of Asp et al (1983) and further characterized by GLC
(Theander & Aman, 1979)

[†] Not analysed

Table 4. Composition and faecal recovery of dietary fibre in carrot
(Mean values and standard deviations)

	Dietary fibre composition* (g/kg dry matter)	Man (g/d)	Intake Rat (g/d)	Faecal excretion	
				Man (%)	Rat (%)
				Mean	SD
Rhamnose + Fucose	23	0.38	0.025	31	36
Arabinose	68	1.84	0.074	3	2
Xylose	10	0.36	0.011	33	32
Mannose	14	0.43	0.015	9	8
Galactose	101	2.31	0.110	3	5
Glucose	246	5.69	0.268	42	31
Sum of neutral sugars	462	11.01	0.503	25	18
Uronic acids	323	8.25	0.352	-	- [†]
Klason lignin	14	0.12	0.015	-	- [†]
Total	799	19.38	0.870	-	- [†]

* Isolated by using the method of Asp et al (1983) and further characterized by GLC
(Theander & Åman, 1979)

[†] Not analysed

Table 5. Composition and faecal recovery of dietary fibre in guar gum
(Mean values and standard deviations)

	Dietary fibre composition* (g/kg dry matter)	Intake		Faecal excretion	
		Man (g/d)	Rat (g/d)	Man (%)	Rat (%)
		Mean	SD	Mean	SD
Rhamnose + Fucose	-	0.10	-	112	60
Arabinose	16	0.38	0.015	5	9
Xylose	-	0.06	-	67	112
Mannose	546	10.50	0.529	0	0
Galactose	325	6.22	0.315	1	2
Glucose	8	0.62	0.008	330	440
Sum of neutral sugars	895	17.88	0.867	13	14
Uronic acids	18	0.30	0.017	-	†
Klason lignin	-	-	-	-	†
Total	913	18.18	0.884	-	†

* Isolated by using the method of Asp et al (1983) and further characterized by GLC (Theander & Åman, 1979)

† Not analysed

‡ Pooled samples from five rats

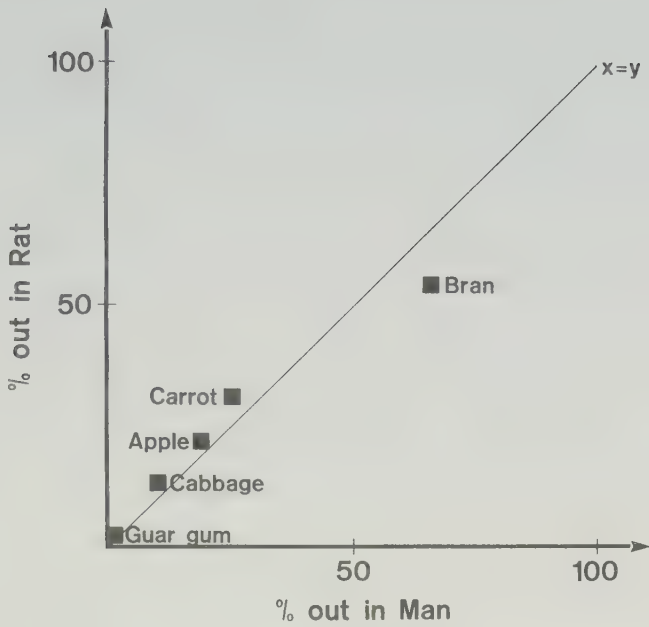


Fig. 1. Faecal recovery of main neutral sugar fibre components.



Fig. 2. Faecal dry weight increment per gram added fibre.

Dietary Fiber Content and Composition in Six Cereals at Different Extraction Rates

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ABSTRACT

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An enzymatic method was used to examine the relationship between extraction rate and dietary-fiber content in flours from wheat, rye, barley, sorghum, rice, and corn. Results were compared with neutral-detergent, acid-detergent, and crude-fiber determinations. Dietary-fiber content in wheat was constant for extraction rates between 65 and 80%, and then increased linearly with the extraction rate. Dietary fiber content in rice and to some extent in barley had a similar relationship, whereas in rye and sorghum it increased gradually with the extraction rate. Soluble dietary-fiber content was constant in each cereal, regardless of the extraction rate, and constituted almost half of the dietary fiber in wheat, rye, and barley

flours of low extraction rate. The relative monomeric composition of the dietary fiber in wheat, rye, and corn was similar at the highest and the lowest rates of extractions, with xylose-arabinose ratios being 0.9-1.5. In barley and rice, the relative xylose content increased with the rate of extraction, whereas in sorghum, both arabinose and xylose content increased. Acid-detergent and crude fiber comprised only a fraction of insoluble and of total dietary fiber. The slope of the regression equation between insoluble dietary fiber and neutral-detergent fiber amylase was 0.8-1.0 for wheat, rye, barley, and rice and 1.2 for corn. Detergent methods applied to sorghum gave obscure results.

Cereal grains are important sources of digestible and unavailable carbohydrates. Cellular areas include the pericarp, testa, germ, aleurone, and endosperm (MacMasters et al 1978, Munck 1981a). Barley and rice kernels are surrounded by a husk (palea and lemma) that, in other cereals, is separated from the kernel during threshing (Barber et al 1981, Munck 1981b). Highly refined white flour consisting mainly of endosperm can be produced if the fibrous outer layers are removed during milling. Thus, dietary-fiber content of flours is determined by rate of extraction.

The analysis of dietary fiber in foods is a controversial subject. Although gas-liquid chromatographic (GLC) and colorimetric methods provide more information, they are too complicated and time-consuming to be used for food labeling and control; thus, for such purposes, gravimetric methods are generally used. In the present study, dietary fiber was measured with a recently developed enzymatic gravimetric method that accounts for both soluble and insoluble components (Asp et al 1983). Soluble dietary-fiber components are quantitatively and qualitatively important in many foods (Asp 1978, Jenkins et al 1976, Stasse-Wolthuis et al 1980). Thus, gel-forming dietary fibers, such as pectins and gums, greatly affect metabolism of carbohydrate and of lipoprotein (Spiller and McPherson-Kay 1980). Cereal fiber has less consistent effects on

metabolism (Asp et al 1981, Brodribb and Humphreys 1976, Jenkins et al 1978, McPherson-Kay and Truswell 1980). Soluble pentosans in wheat and rye affect baking properties (Ciaccio and D'Appolonia 1982). Water-soluble β -glucans in barley are important quantitatively and influence feed utilization (Burnett 1966).

In the present investigation, variation of insoluble and soluble dietary fiber with the rate of extraction of flour from wheat, rye, barley, sorghum, and rice was studied. Different types of corn flour were also studied. The composition of dietary fiber was determined by GLC in flours having the lowest and the highest extraction rates. Finally, crude fiber, neutral-detergent fiber (NDF) (with amylase), and acid-detergent fiber (ADF) measurements were compared with determinations obtained by the enzymatic-gravimetric method.

MATERIALS AND METHODS

Materials

Hard winter wheat, rye, and spring barley harvested in Denmark in 1980 were used. Sorghum and rough rice from Italy were obtained from commercial sources. The cereals were milled into flours with extraction rates between 100 and 65%. Different types of corn flours were produced from U.S. No. 3 corn.

Wheat and rye flours were prepared by laboratory roller mills (Quadrumat Junior and Quadrumat Junior II, Brabender GmbH, Duisburg, BRD), and by sifting (Laboratory Plansifter, Bühler-Miag, Braunschweig, BRD), as described by Pedersen and Eggum (1983). Flours of barley were prepared by abrasive milling (Vertical Abrasive Polisher, Schule GmbH, Hamburg, BRD). Sorghum flours were produced by decortication (DVA decorticator, United

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Milling System A/S, Copenhagen, Denmark) and by sifting, as described by Munck et al (1982). Brown rice was prepared by passing rough rice through a Strong Scott Barley Peeler (Seedburo Equipment Co., Chicago, IL). Unshelled rough rice was removed by hand, and the milling yield of brown rice was 82%. Milled rices were prepared by decortication (DVA decorticator, UMS) and by abrasive milling (Vertical Abrasive Polisher, Schule). Milling yields, calculated on basis of rough rice, were 77, 72, 68, and 64%. Various types of corn flour were produced by dehulling (Dehuller DHA 400, UMS) after tempering with 4–5% distilled water for 10 min, disk-milling (MHA 400, UMS), and sifting (Laboratory Plansifter, Bühler-Miag). Corn flours with particle sizes of $<340\ \mu$, $<840\ \mu$, and 500–1,000 μ were prepared in this way. Degerminated corn grits were produced by gravity separation (separator type 51, Cimbria, Thisted, Denmark) of the dehulled corn. The whole grains and the cereal products were finally hammer milled (Laboratory Mill, Christy & Norris Ltd., UK).

Methods

Enzymatic gravimetric method. The total dietary-fiber content was analyzed by a gravimetric method based on digestion of the sample with enzymes, as described by Asp et al (1983). The dietary fiber can be classified into insoluble and soluble components, the sum of which is the total dietary fiber. This method was developed from that of Hellendoorn et al (1975), with the use of the physiological enzymes pepsin (Merck, no. 7190) and pancreatin (Sigma, no. P-1750). Pepsin incubation is performed at pH 1.5 for 1 hr and pancreatin incubation at pH 6.8 for an additional 1 hr. Starch digestion is improved when a heat-resistant α -amylase (Termamyl, Novo, Copenhagen) is added during the initial 15-min gelatinization. Insoluble components are recovered by filtration. Soluble components are recovered by precipitation with four volumes of 95% ethanol and filtration. The results are corrected for undigested protein (Kjeldahl $N \times 6.25$) and ash (ignition at 525°C for 24 hr) associated with the fiber.

Other gravimetric methods. Crude fiber was determined according to AOAC methods (1980). ADF and NDF were analyzed as described by Goering and Van Soest (1970). The NDF determinations were performed with the following modifications: the cereal was suspended in a phosphate-buffer, pH 7.0, containing 2 g of α -amylase (Merck) per 500 ml of phosphate-buffer. The suspension was incubated for 15 hr at 37°C . To improve the starch digestion further, 100 μL Termamyl (60 L) was added during NDF incubation.

Gas-chromatographic determinations. The dietary-fiber composition was assayed by GLC for the neutral sugars obtained

after acid hydrolysis and by a decarboxylation method for the uronic acids, as described by Theander and Åman (1979). The hydrolysis was performed with 72% (12.0 M) sulfuric acid (5 ml per 100 mg) at room temperature for 2 hr, diluted with water to 0.358 M sulphuric acid, and refluxed for 6 hr. The monomeric composition was determined only for the lowest and the highest extraction rates of each cereal. Soluble and insoluble dietary-fiber components were pooled for these analyses. All dietary-fiber components are expressed as polysaccharides, ie, constituting monomers are expressed as anhydroform.

Starch determination. Approximately 20 mg of the dietary fiber was suspended in 1 ml of distilled water and gelatinized for 15 min. Five microliters of amyloglucosidase (Boehringer Mannheim GmbH) was added, and the suspension was incubated at 60°C for 30 min. The released glucose was measured with glucose oxidase reagent (Glox-Novum, Kabi Diagnostica, Stockholm, Sweden).

All values are given in percent on a moisture-free basis and represent means of duplicate analyses.

RESULTS

Wheat

The dietary-fiber content of wheat flours ranged from 2.8 to 12.1% (Fig. 1). The soluble-fiber content was about 1.3%, independent of the degree of extraction. The content of insoluble fiber increased rapidly for extraction rates above 80%. The relative composition of the monosaccharides differed little between the two extraction rates investigated. The main components were glucose, arabinose, and xylose (Table I).

By the NDF-amylase method, approximately 70% of the total fiber and 93% of the insoluble fiber obtained with the enzymatic method can be measured (Table II). ADF and crude fiber versus insoluble fiber with the enzymatic method had intercepts at 0.7–0.8.

Rye

Rye contained from 7.5 to 16.1% dietary fiber (Fig. 2). The soluble-fiber content was about 3.8% at all extraction rates. The content of insoluble fiber increased continuously with the degree of extraction. The relative monomeric composition was similar in the flours of highest and lowest extraction. The relative distribution of monomeric sugars was similar to that in wheat (Table I).

Correlation of total fiber in rye with NDF, ADF, and crude fiber values showed intercepts indicating the large amount of soluble fiber (Table II). The NDF-amylase method gave values of about 80% of the insoluble fiber assayed enzymatically.

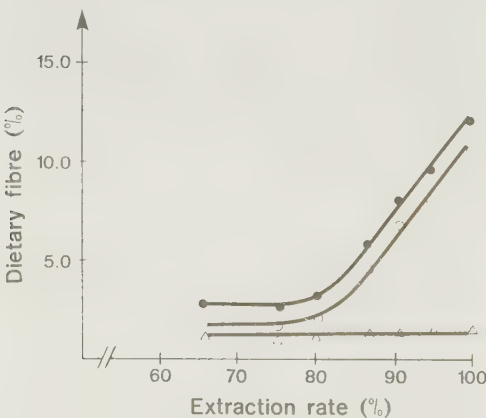


Fig. 1. Dietary fiber content in wheat flours of different extraction rates. ● = total dietary fiber; ○ = insoluble dietary fiber; △ = soluble dietary fiber.

TABLE I
Composition of Dietary Fiber in Wheat, Rye, and Barley

	Extraction Rate (%)					
	Wheat		Rye		Barley	
	66	100	66	100	69	100
Polysaccharides (g/100 g)	2.4	9.5	6.8	12.5	7.9	15.1
Relative composition (%)						
Rhamnose	...	...	...	...	...	...
Arabinose	24	24	25	25	12	14
Xylose	34	36	31	37	14	26
Mannose	4	1	5	3	4	3
Galactose	7	3	3	3	1	1
Glucose	31	33	31	28	69	51
Uronic acids	...	3	5	4	...	5
Klason lignin (g/100 g)	...	2.0	0.3	2.1	...	3.5
Sum of dietary fiber components (g/100 g)	2.4	11.5	7.1	14.6	7.9	18.6
Dietary fiber residue*	2.8	12.1	7.5	16.1	8.2	18.8

*With the gravimetric method (sum of insoluble and soluble fraction, g/100 g).

TABLE II
Regression Equations and Correlation Coefficients Between Total Dietary Fiber
and Insoluble Dietary Fiber Obtained with the Gravimetric Method (X)
and Neutral-Detergent, Acid-Detergent, and Crude Fiber (Y)

Grain	Total Dietary Fiber		Insoluble Dietary Fiber	
	Regression Equations	Correlation Coefficient (r)	Regression Equations	Correlation Coefficient (r)
Wheat	$Y = 0.88X - 0.82^a$	0.98	$Y = 0.93X + 0.06^a$	0.98
	$Y = 0.35X - 0.63^b$	0.99	$Y = 0.37X - 0.29^b$	0.99
	$Y = 0.25X - 0.42^c$	0.99	$Y = 0.26X - 0.18^c$	0.99
Rye	$Y = 0.75X - 2.31^a$	0.98	$Y = 0.79X + 0.12^a$	0.98
	$Y = 0.27X - 1.01^b$	0.98	$Y = 0.28X - 0.12^b$	0.98
	$Y = 0.15X - 0.15^c$	0.97	$Y = 0.16X + 0.36^c$	0.96
Barley	$Y = 0.97X - 3.17^a$	0.95	$Y = 0.99X + 0.44^a$	0.95
	$Y = 0.42X - 2.7^b$	0.99	$Y = 0.43X - 1.1^b$	0.98
	$Y = 0.39X - 2.6^c$	0.98	$Y = 0.39X - 1.1^c$	0.97
Sorghum	$Y = 0.13X + 7.2^a$	0.72	$Y = 0.13X + 7.23^a$	0.71
	$Y = 0.43X + 5.25^b$	0.91	$Y = 0.44X + 5.44^b$	0.90
	$Y = 0.20X + 0.28^c$	0.99	$Y = 0.21X + 0.35^c$	0.99
Rice	$Y = 0.94X - 0.59^a$	1.00	$Y = 0.95X - 0.03^a$	1.00
	$Y = 0.75X - 0.22^b$	1.00	$Y = 0.76X + 0.66^b$	1.00
	$Y = 0.67X - 0.69^c$	1.00	$Y = 0.67X - 0.3^c$	1.00
Corn	$Y = 1.29X - 1.52^a$	0.99	$Y = 1.21X - 0.62^a$	0.99
	$Y = 0.25X - 0.27^b$	0.92	$Y = 0.24X - 0.12^b$	0.93
	$Y = 0.22X - 0.06^c$	0.88	$Y = 0.21X + 0.07^c$	0.90

^a Obtained with neutral detergent.

^b Obtained with acid detergent.

^c Obtained with crude fiber.

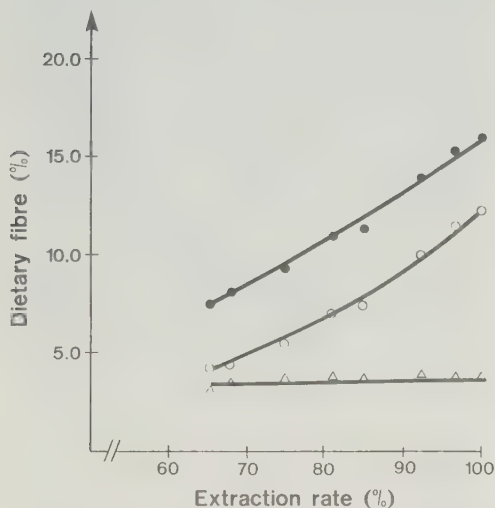


Fig. 2. Dietary fiber content in rye flours of different extraction rates. ● = total dietary fiber; ○ = insoluble dietary fiber; △ = soluble dietary fiber.

Barley

Barley contained 8.2% dietary fiber at the lowest extraction rate and 18.8% in the whole grain (Fig. 3). The soluble fraction was about 3.9% at all extractions. The relative pentosan content was lower and the glucan content higher in barley than in wheat and rye. The monomeric composition between the two extraction rates analyzed also differed (Table I). The main components were glucose, arabinose, and xylose. The relative arabinose content was similar, but the xylose content increased, and the glucose content decreased with increasing extraction rates.

NDF-amylase values correlated well with insoluble fiber. As in rye, ADF and crude fiber values were very similar (Table II).

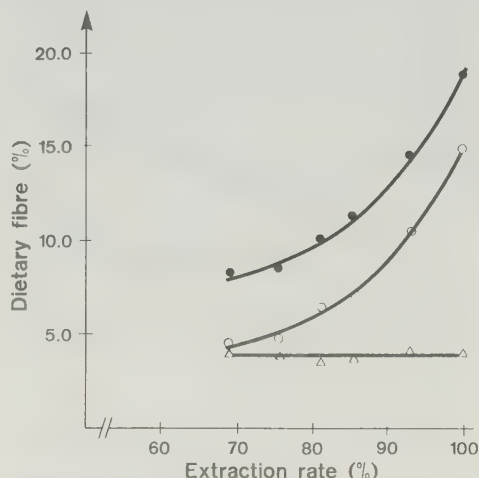


Fig. 3. Dietary fiber content in barley flours of different extraction rates. ● = total dietary fiber; ○ = insoluble dietary fiber; △ = soluble dietary fiber.

Sorghum

Sorghum contained only 2.5–9.0% total dietary fiber, and the soluble fraction was about 0.5% in all flours (Fig. 4). The increase in the insoluble fraction was almost proportional to the extraction rate. The dietary fiber in sorghum flour of low extractions consisted mainly of glucans. At high extraction rates, the proportion of pentosans (arabinose and xylose) increased. Uronic acids constituted 11–13% of the dietary fiber in sorghum flours (Table III).

NDF as well as ADF values of sorghum flours were higher than both total and insoluble fiber with the enzymatic method (Table II). The difference between ADF and NDF values and those found by the enzymatic methods were most pronounced in flours of low extraction rates. Crude fiber values correlated well with both total

and insoluble fiber determined by the enzymatic method and gave about 1/5 of the total fiber.

Rice

Rice contained from 0.7 to 19.2% total dietary fiber. Removal of the husk, corresponding to about 80% extraction rate, decreased the dietary fiber content to 2.9%. The soluble-fiber fraction was less than 1.0% at all extraction rates (Fig. 5). The main dietary-fiber components in the most refined rice were glucose and uronic acids. In rice flour, including the husk, there were also appreciable amounts of xylose (Table III).

Because rice contains a very small amount of soluble fiber, there was good correlation between NDF-amylase values with both total and insoluble fiber assayed enzymatically (Table II). ADF and crude-fiber values were comparatively high because cellulose is a dominating component in dietary fiber from rice (Table III).

Corn

The total dietary fiber in corn varied from 3.9 to 9.3%. The soluble fraction was small, approximately 0.5%, in all flours (Fig. 6). The chemical composition was similar in whole corn and in the fine corn flour (particle size $<340\ \mu$) analyzed. The main components were glucose, arabinose, and xylose (Table III).

NDF-amylase values correlated well with total and insoluble

fiber obtained by the enzymatic method, although NDF-values tended to be slightly higher (Table II).

Acid-Insoluble Lignin

Lignin, measured as Klason lignin (acid-insoluble residue), was not detectable or was very low in the most refined flours (Tables I and III). The highest values, 3.5 and 3.9%, were found in whole grain of barley and rice, respectively.

Residual Starch in the Dietary-Fiber Residue

Starch, measured with glucose oxidase after incubation with amyloglucosidase, was not detectable or was very low ($\leq 0.1\ \text{g}/100\ \text{g}$ of flour) in the dietary fiber fractions of all flours investigated.

Comparison of Gravimetric and Gas-Chromatographic Dietary Fiber Assay

As shown in Tables I and III, the sum of the analyzed polysaccharides (measured with GLC) and Klason lignin correlated

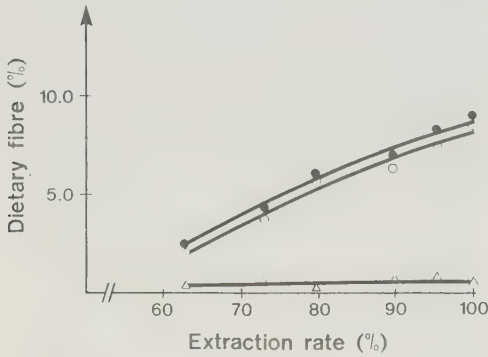


Fig. 4. Dietary fiber content in sorghum flours of different extraction rates. ● = total dietary fiber; ○ = insoluble dietary fiber; △ = soluble dietary fiber.

TABLE III
Composition of Dietary Fiber in Sorghum, Rice, and Corn

	Extraction Rate (%) of					
	Sorghum		Rice		Corn	
	63	100	64	100	Fine	Whole
Polysaccharides (g/100 g)	2.3	6.7	1.1	13.0	4.1	8.0
Relative composition (%)						
Rhamnose	---	---	---	---	---	---
Arabinose	3	18	4	6	26	24
Xylose	5	15	4	25	24	30
Mannose	9	3	5	1	2	2
Galactose	9	2	5	2	5	5
Glucose	61	51	72	57	30	32
Uronic acids	13	11	10	9	13	7
Klason lignin (g/100 g)	0.4	2.5	---	3.9	0.8	1.4
Sum of dietary fiber components (g/100 g)	2.7	9.2	1.1	16.9	4.9	9.4
Dietary fiber residue ^a	2.5	9.0	0.7	19.2	3.9	9.3

^aWith the gravimetric method (sum of insoluble and soluble fractions, g/100 g).

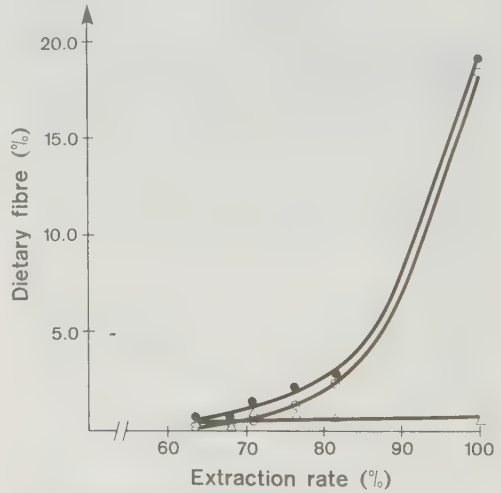


Fig. 5. Dietary fiber content in rice flours of different extraction rates. ● = total dietary fiber; ○ = insoluble dietary fiber; △ = soluble dietary fiber.

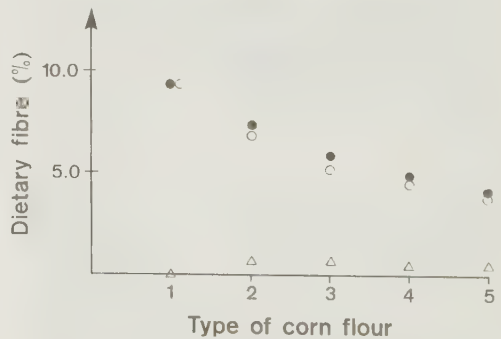


Fig. 6. Dietary fiber content in different corn products. 1, whole corn; 2, 500–1,000 μ corn flour; 3, $<840\ \mu$ corn flour; 4, $<340\ \mu$ corn flour; 5, dechulled and degerminated corn flour. ● = total dietary fiber; ○ = insoluble dietary fiber; △ = soluble dietary fiber.

well with the total dietary fiber (corrected for ash and undigestible protein) determined by the enzymatic method. The correlation coefficient was 0.99. The lower values obtained with GLC in some samples may have resulted from undetermined minor components such as phenolic acids (Smart and O'Brien 1979) or from losses during hydrolysis, before GLC. The higher values in other samples might be caused by overlapping of the undigestible protein corrected for in the enzymatic method and by Klason lignin in the GLC method (Robertson and Van Soest 1981, Theander and Aman 1981).

DISCUSSION

As expected, the cereals analyzed showed increased dietary fiber content with increasing extraction rate. The slope and the start and endpoints of the curves differed, however. These differences are explained by differences in histological constitution of the whole grains, as well as by differences in the degree of separation of the various cell layers obtained in the dry-milling process. Thus, the constant dietary-fiber level below 80% extraction rate and the sharp rise in insoluble dietary fiber above 80% extraction rate in wheat shows that the pericarp, testa, and aleurone are better separated from the endosperm in this cereal than in rye. The same tendency is seen in barley and rice compared to sorghum when all three cereals are subjected to milling. Furthermore, barley and rice contain husks that are rich in insoluble dietary fiber and lignin (Barber et al 1981, Munck 1981a, Salomonsson et al 1980). This explains the higher concentration of nonstarchy polysaccharides and total dietary fiber in these two cereals.

A critical step in all dietary-fiber methods is the removal of starch. Starch residues add to the dietary fiber and appear as glucose in gas-chromatographic determination of dietary-fiber composition. The combination of Termamyl and pancreatin in the enzymatic gravimetric method used here leaves almost no starch residues in unprocessed flours (Asp et al 1983). All dietary-fiber residues obtained were also checked for residual starch by amyloglucosidase incubation, and the highest value found was 0.1% (dry basis). Resistant starch (ie, starch available to amylase only after solubilization with 2M KOH) has been reported to occur in the dietary-fiber fraction of processed foods (Englyst et al 1982). Resistant starch has not been demonstrated in unprocessed flours, nor did we find measurable amounts of it when analyzing wheat and rye flours by the enzymatic gravimetric method (*unpublished results*). Further evidence for the efficiency in starch removal is the agreement between dietary-fiber glucan values in the present study and those reported by Englyst et al (1982) for wheat, rye, and barley.

The amount of soluble fiber was higher in rye, barley, and wheat than in the other cereals. The water-soluble fraction in wheat and rye contains mainly arabinoxylans (Geissmann and Neukom 1973, Holas et al 1972) whereas in barley it is dominated by β -glucans (Fincher 1975). In all the cereals we investigated, the content of soluble fiber was independent of the extraction rate, showing that soluble polysaccharides arise essentially from the endosperm. Our results for wheat are in accordance with those reported by Neukom et al (1977), who found 0.8–1.2% soluble, ethanol precipitable, nonstarchy polysaccharides in wheat flours of various extraction rates. Only traces of nonstarchy polysaccharides (0.02–0.05%) were not precipitated by ethanol. The total nonstarchy polysaccharide content was 2.2–10.3%, similar to the results reported in Table I. The higher pentosan content of wheat and rye is probably important for its baking properties, especially certain soluble pentosan fractions (Ciaccio and D'Appolonia 1982).

In wheat and rye, the monomeric composition of the dietary fiber was similar at high and low extraction rates. This suggests that the xylose-arabinose ratio in the endosperm and outer layers is similar. Our results for wheat are in agreement with those of Englyst et al (1982). The monomeric composition of nonstarchy polysaccharides in rye and barley reported by these authors also agrees with our results. Anderson and Clydesdale (1980), however, found a higher xylose-arabinose ratio in wheat. According to Medcalf et al (1968) and Wilson Lee and Stenvert (1973), the proportions between

arabinose and xylose vary in different wheat varieties.

In barley and rice, the relative proportion of xylans increased with increasing extraction rate, whereas that of glucans decreased. The kernels of these cereals are surrounded by a husk, which, in barley, is rich in xylans (Salomonsson et al 1980). This explains the dramatic rise in xylose content in the flours containing the husk (100% extraction rate). The increase in xylose content at high extraction rate in barley and rice is in contrast to that found in wheat, rye, and corn. These cereals had relatively uniform arabinose-xylose ratio at the various extraction rates. According to Fincher (1975) endosperm cell walls of barley contain up to 75% of mixed β -glucans. This, together with the removal of the aleurone cells consisting mainly of arabinoxylan carbohydrates (McNeil et al 1975), explains the relative increase in glucose-based dietary fiber constituents at the low extraction rate.

In sorghum, both the arabinose and xylose content was higher in whole grain than in refined flour. This is in agreement with Wooldar et al (1977), who found that the outer layers contained an arabinoxylan.

The fine corn flour had a xylose-arabinose ratio around 1. The tendency to higher xylan and glucan content in the whole grain flour is in agreement with the high cellulose and xylan content in corn bran (Schimberni et al 1982).

Appreciable amounts of uronic acids were found only in sorghum, rice, and corn. The presence of pectic substances in rice endosperm cell walls has been reported (Shibuya and Iwasaki 1978). The absence of pectins in wheat and barley endosperm is in agreement with results from studies by Fincher (1975) and Mares and Stone (1973).

The degree of lignification differed considerably with the rate of extraction, reflecting the fact that only the pericarp and husk are lignified. This may be important for physiological properties, such as resistance to bacterial fermentation in the large intestine, and fecal bulking (Cummings 1981).

The general appearance of the variation in the dietary fiber in relation to extraction rate is probably typical. Analyses of another variety of, for example, wheat, might change the start and end points of the curves slightly. The milling procedure used may also affect the slope of the curves. Nevertheless, the determination of dietary fiber by gravimetric analyses can probably be useful for prediction of extraction rate of flours used in cereal products. In processed products to which salt has been added, ash determination cannot be used for estimation of extraction rates.

Comparisons of different gravimetric dietary-fiber methods show that the NDF-amylase procedure correlates differently with total dietary fiber in different cereals. This is mainly because the content of soluble dietary-fiber components varies. In wheat, rye, and barley of low extractions, almost half of the total dietary fiber was soluble, clearly demonstrating that this fraction should be included in a proper assay of dietary fiber.

The NDF amylase method seems to solubilize some rye components that are insoluble with an enzymatic method. ADF values and crude-fiber values correlate differently with total and insoluble fiber in different cereals. Earlier investigations of the relationship between extraction rate and fiber content used the crude-fiber method (Ziegler and Greer 1978). By this procedure, which measures only a small and variable part of dietary fiber, fiber traces of 0.1% have been found in white wheat flour and of 2.2% in whole grain wheat flour. Thus, the underestimation of dietary fiber by this method is most pronounced in flour of low extraction, and therefore a comparison of crude fiber values in various flours would be misleading.

NDF and ADF determination in sorghum implies special problems related to proteins that are difficult to solubilize (Bach Knudsen and Munck 1983). Because a low-tannin sorghum was used in the present study, tannin is unlikely to have interfered.

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Fermentation of dietary fibre in the intestinal tract of rats
A comparison of flours with different extraction rates
from six cereals

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SUMMARY

The fermentation of dietary fibre in wheat, rye, barley, sorghum, rice and maize was investigated in balance experiments with rats. Two different extraction rates, 100% and approximately 65% were investigated for each cereal grain. In the case of maize, whole maize and dehulled maize, ground and sieved so that it contained mainly endosperm, was investigated. Except for sorghum, dietary fibre in low-extraction flours was more fermented than in whole-grain flours. The relative monomeric composition of the fibre at low and high extraction rate in wheat and rye was similar, but the susceptibility to fermentation by bacterial enzymes was quite different. This indicates that a high proportion of soluble fibre and a low content of lignin improves the fermentability of the fibre. Further, barley, rice, and sorghum fibre in refined flours, all consisting of mainly non-lignified glucans fermented more extensively when the proportion of soluble fibre was high. Cellulose addition to diets containing wheat flour did not change the susceptibility of the wheat fibre to bacterial fermentation. Starch was demonstrated in faeces from rats fed wheat, sorghum and whole-grain rye flours. This starch represented 17-61% of the total faecal glucan, but constituted less than 1% of the starch intake.

INTRODUCTION

Lately, an increasing interest in the consumption of dietary fibre has developed. The most important source for human nutrition is cereal grains, representing 40% of the average dietary fibre intake in Sweden¹. Micro-organisms, present in the large intestine, ferment dietary fibre to various extents². The physical and chemical structure and solubility of the fibre are factors affecting the susceptibility to bacterial fermentation^{3,4}.

Soluble dietary fibre is more easily fermented than insoluble dietary fibre⁵. In a recent study we have shown that the soluble fibre fraction in some cereals is quantitatively important, especially in flour with low extraction rates⁶. Further the degree of lignification was highly dependent on the extraction rate and, according to Chandler et al.⁷ lignin impaires the fermentation of other cell-wall polysaccharides. Bertrand et al.⁸, however, did not find any increase in the degree of fermentation after delignification of wheat bran, in rats.

Dietary fibre resistant to bacterial degradation has a good bulking capacity, while that of easily fermented dietary fibre is poor. However, dietary fibre susceptible to fermentation, can be utilized as energy by the intestinal flora^{9,10} and perhaps also by man¹¹. The extent of fermentation might also influence mineral and vitamin absorption in the colon. The importance of fermentation in relation to vitamin absorption was demonstrated by Rotenberg et al.¹². Pectin, which is an easily fermented dietary fibre, increased the bacterial activity and stimulated the synthesis of niacin, thiamin and riboflavin in the large intestine, in rats. James et al.¹³ suggested that calcium might be absorbed from the colon when released from dietary fibre through fermentation. Consequently, the extent of fermentation is important for several physiological properties of dietary fibre³.

The dietary fibre content in flour from cereals depends on the extraction rate. During milling to produce refined flours the outer fibrous layers are removed and the dietary fibre content decreases with decreasing extraction rate⁶. A higher extraction rate means more hull and probably a modified chemical structure of the dietary fibre which

might result in a different degree of fermentation.

The present investigation was performed to compare the extent of fermentation and faecal bulking capacity of dietary fibre from different cereal grains through balance experiments in rats. Because the recent interest on the digestibility of starch, the faeces was also analysed considering a possible presence of α -glucans. Furthermore, the effect of cellulose addition in relation to fermentation of other fibre components was evaluated.

EXPERIMENTAL

Materials

Wheat, rye, barley, rice, sorghum and maize were investigated. Flours with two different extraction rates, 100% and approximately 65%, were investigated for each cereal, except maize. In the case of maize, whole maize and maize sieved to a small particle size ($<340\mu$) were analysed. The fine-maize fraction ($<340\mu$) consisted mainly of endosperm¹⁴. The preparation of the different flours has been described by Nyman et al.⁶.

Methods

All analyses were done in duplicate and the results are given on a moisture-free basis.

Dietary fibre. The dietary fibre content in the cereals was analysed gravimetrically and separated into soluble and insoluble fibre, after digestion with physiological enzymes, using the method of Asp et al.¹⁵. The dietary fibre composition of cereals and faeces was determined using gas-liquid chromatography for neutral sugars¹⁶, allose was used as internal standard. Uronic acids were measured with a decarboxylation method¹⁶. All dietary fibre components were expressed as polysaccharides i.e. monomer weight $\times 0.9$. The hydrolysis was performed with 72% (12M) sulphuric acid (2 ml per 100 mg) at room temperature for 2 h. The sulphuric acid was then diluted with water to 0.36 M and refluxed for 6 h¹⁶. The residue insoluble in 72% H_2SO_4 was defined as 'Klason lignin'.

Starch determination. Approximately 100 mg of faeces were suspended in distilled water (1ml) and gelatinized for 15 min. in a boiling water bath. Amyloglucosidase (5 μ l, 14U/mg, Boehringer Mannheim GmbH) was added and the suspension was incubated, by shaking every tenth minute, at 60°C for 30 min. The released glucose was measured with glucose oxidase/peroxidase reagent (Glox-Novum, Kabi Diagnostica, Stockholm, Sweden). Starch values were expressed as glucose content $\times 0.9$.

In some experiments 5 μ l of a thermostable α -amylase (Termamyl, 120L, Novo A/S, Denmark) was added in the gelatinization step

above^{17,18}. However, we obtained the same results for starch content, with- or without the addition of Termamyl.

Animals and diets

The experimental procedure has been described in detail by Eggum¹⁹. Male Wistar rats weighing approximately 75 g were divided into groups of five. Each animal received 150 mg N and 10 g dry weight per day. Water was provided ad lib. After a 4 d adaptation period, feed residues and faeces were collected during a 5 d balance period. A 4 d adaptation period to the diet has been shown to be enough for evaluation of the fermentability of fibre in rats²⁰ and also for evaluation of the protein nutritional value¹⁹. The faeces were collected daily, stored at -20°C and lyophilized. Faecal dry weights were measured on samples collected from individual rats. The carbohydrate analyses (neutral sugars, uronic acids and starch) were performed on proportionally pooled samples from five rats. Feed and cereal fibre intake, faecal dry weights, and dry matter digestibilities are shown in Table I.

To adjust the dietary N concentration, two N-free mixtures were used, one with and the other without cellulose. The N-free mixture with cellulose contained (in g/100g dry matter): sucrose (10), cellulose (5), maize oil (5) and autoclaved potato starch (80). The N-free mixture without cellulose contained the same proportions of sucrose and maize oil but 85 g of autoclaved potato starch.

Flours of different extraction rates from wheat, rye, barley, sorghum, rice and maize were used as the only protein source in the cereal diets at a concentration of 1.5% N (dry matter). Normally the N-free mixture with cellulose was used when adjusting dietary N content. The N-free mixture without cellulose was applied in two wheat diets, refined and whole-grain wheat flour, and three casein-based diets (1.5% N dry matter), supplied with 0, 0.7, and 2.0% cellulose.

All diets contained 3.2% of a mineral mixture and 1.6% of a cellulose-free vitamin mixture¹⁹. The vitamin mixture included 16.7% choline chloride.

Calculation. Dietary fibre constituents excreted in faeces, were corrected for the basal excretion, i.e. the excretion of saccharides occurring in faeces when the rats were fed a fibre-free casein diet. In addition, when the cereal diets contained purified cellulose, the faecal excretion of glucans was corrected for 82% of added cellulose, i.e. the fraction of this cellulose determined to be non-fermentable (see Results). Faecal glucose values, were also corrected for the small amounts of free glucose present (on average 10 mg/5d). The term glucan refers to any polymer of glucose, both α -glucans and β -glucans.

Statistical evaluation. When determinations were performed on samples collected from individual rats, Student's t-test was used in the statistical evaluation. When analyses were performed on pooled samples a variation coefficient (the quotient between the standard deviation and the mean value) $\leq 30\%$ was assumed and then Student's t-test was applied. This correlation coefficient is considerably higher than that earlier obtained in experiments with cereals on individual rats (i.e. 4% for wheat bran⁴, 12% for whole-grain wheat flour and 9% for refined wheat flour, M.Nyman and N.-G.Asp, unpublished results).

RESULTS

Bacterial degradation of dietary fibre in the intestinal tract of rats.

Casein-based diets

Basal diet. The main carbohydrate in this diet was autoclaved potato starch. No dietary fibre was added. The faecal excretion of typical dietary fibre constituents by rats fed autoclaved potato starch was low, i.e. (in mg/5d): rhamnose (10), arabinose (3), xylose (6), mannose (2), galactose (13), glucose (34) and uronic acids (5). These values are similar to those reported in earlier studies with raw wheat starch and raw maize starch^{4,18}. No starch, measured as glucose after incubation with amyloglucosidase, could be detected in the faeces.

Basal diet with 0.7 or 2.0 % cellulose. The cellulose added was a relatively pure glucan (93%) with small amounts of xylose (3%), mannose (2%) and galactose (2%). The fraction of cellulose excreted in faeces, was very similar for both the 0.7 and 2.0% cellulose diets, 81 and 83%, respectively. The faecal excretion of the minor components, rhamnose, arabinose, xylose, mannose, galactose and uronic acids, was approximately as low as on the basal diet, without added cellulose. No starch could be detected in the faeces.

Cereal-based diets

The dietary fibre content and composition of the different cereals have been described in detail by Nyman et al.⁶. The fibre intake of the various cereals are reported in Table I.

Faecal dry weights. Whole-grain flours caused, without exception, significantly ($p < 0.001$) higher faecal dry weights than the corresponding refined flours. Faecal dry weights obtained with whole-grain wheat, sorghum and maize flours were similar. Whole-grain rye produced still higher faecal dry weights ($p < 0.001$) and those with rice and barley were significantly ($p < 0.001$) higher than with rye (Table I).

Concerning the refined flours, the faecal bulking capacity with rye was significantly ($p < 0.05$) higher than with the other cereals. Rice, however, caused a significantly lower faecal dry weight ($p < 0.001$), which was the same as with the basal diet.

The faecal dry weights obtained in relation to dietary fibre intake were different. Thus, although the dietary fibre content in refined rye and barley flour was similar⁶, the faecal dry weight obtained with refined rye flour in the diets was significantly ($p < 0.05$) higher than that obtained with barley flour. Furthermore, no significant differences in faecal dry weights were obtained after feeding refined barley and fine maize flour, despite the fact that the dietary fibre intake was 70% higher with refined barley than with fine maize flour in the diet.

Wheat. The main dietary fibre constituents in refined flour were polymers containing arabinose, xylose and glucose and, in whole-grain flour, lignin also was an important constituent (Table II). The relative monomeric composition of the saccharides was similar in refined and in whole-grain flour⁶.

Wheat of low extraction was fermented to a higher degree than whole-grain flour, 24 and 62 % of the total dietary fibre content, respectively, being excreted in faeces ($p < 0.01$).

The faecal recovery of the arabinose and the xylose in refined wheat flour was similar and constituted about 10% of the intake. The glucans were less degraded and 69% of glucose of the non-starch polysaccharide in the diet appeared to be excreted in faeces. However, a large amount (61%) of the faecal glucan was in fact starch, although the faecal starch constituted less than 1% of the dietary starch. After correction for remaining starch in faeces the faecal recovery of β -glucans (i.e. non-starch glucans) was found to be 29%.

Arabinose- and xylose-based fibre in whole-grain wheat flour were relatively resistant to bacterial degradation and approximately 45% was excreted in faeces. Also, with whole-grain wheat flour, a considerable amount of the excreted total glucan was starch (27%). After correction for remaining starch in faeces it was found that 64% of the dietary β -glucans was excreted.

A comparison of the fermentability of the main components of the dietary fibre in wheat flours with and without purified cellulose in the diet showed similar results. Excretion of starch was also observed with diets containing no cellulose addition. With refined wheat flour in the diet 39% of the excreted glucan was starch, and with whole grain wheat flour the corresponding value was 32%.

Rye. The main dietary fibre components were polymers containing arabinose, xylose and glucose (Table III). The relative monomeric composition was very similar in the two rye flours, and also similar to that in wheat⁶. The faecal recovery of total dietary fibre components was 36% in refined flour and 56% in whole-grain flour, and the difference was significant ($p < 0.05$).

With whole-grain rye flour in the diet, 19% of the excreted glucans was due to starch. With refined rye flour, only minute amounts of starch were detected in the faeces.

Barley. Barley fibre mainly consisted of β -glucans and considerable amounts of polymers containing arabinose and xylose (Table IV). Whole-grain barley was highly lignified. The relative xylose content increased and the relative glucose content decreased at the high extraction rate⁶.

The main dietary fibre constituents in barley showed similar availability to micro-organisms. The faecal excretion of the main components arabinose, xylose and glucose, averaged 15% in refined flour versus 63% in whole-grain flour. The percent faecal excretion of total fibre was significantly ($p < 0.001$) lower with refined flour.

The faecal excretion of starch with the barley flours was small in the relation to the excreted glucans and represented approximately 5%.

Sorghum. Low-extraction-rate sorghum fibre was a rather pure β -glucan, which was highly resistant to bacterial fermentation, 82% being excreted in the faeces (Table V). The dietary fibre in the high-extraction-rate preparation contained, in addition to glucose, considerable amounts of arabinose, xylose and lignin. The dietary fibre constituents in whole-grain flour seemed to be more extensively degraded, only 45% of the main components, arabinose, xylose and glucose,

being excreted in faeces.

The faecal excretion of starch in percent of excreted glucans was 17 and 26% with refined and whole-grain sorghum flour, respectively, corresponding to approximately 0.5 and 1% of the dietary starch.

Rice. Flour of low extraction rate contained a rather pure β -glucan fibre, which was almost completely fermented, only 8% being excreted in faeces (Table VI). The fibre in the high-extraction-rate preparation, including the husk, also contained considerable amounts of xylose and lignin, which were highly resistant to bacterial fermentation. The fermentation of whole-grain flour was significantly ($p < 0.001$) lower than that of refined flour. No starch could be detected in the faeces.

Maize. The main dietary fibre components were arabinose, xylose, glucose and lignin (Table VII). The relative monomeric composition of the polysaccharides was similar in both flours investigated and also similar to that in wheat and rye⁶.

The fibre in maize, milled and sieved to a small particle size consisting mainly of endosperm¹⁴, was fermented to a significantly ($p < 0.05$) higher degree than fibre in whole maize. The faecal excretion of the main dietary fibre constituents, arabinose, xylose and glucose, was 32% with fine maize flour and 63% with whole maize flour. Only minute amounts of starch could be found in the faeces of rats fed the maize flours.

DISCUSSION

Dietary fibre in refined flour was, in general, fermented to a higher degree than fibre in whole-grain flours. The breakdown of the dietary fibre constituents in wheat, rye and maize differed markedly between the two extraction rates investigated, although the relative monomeric composition of the dietary fibre was similar in endosperm and outer layers⁶. The soluble fibre components, however, constituted a much larger fraction of the total dietary fibre at low extraction rates, and in general soluble dietary fibre is more easily fermented than insoluble fibre³⁻⁵. Furthermore, only the pericarp and husk are lignified and, according to Chandler et al.⁷, lignin is a physical hindrance for bacterial breakdown of fibre. Bertrand et al.⁸ however, found similar degradation of untreated and chemically delignified wheat bran in the rat intestine. In our study the amount of soluble fibre seems to be of significant importance for the fermentation of the fibre. Dietary fibre in refined rice and sorghum flours, and to some extent in refined barley flour, consisted mainly of β -glucans having no or very small amounts of lignin. Nevertheless, the degree of fermentation was quite different. In sorghum, with a very small amount of soluble fibre (15%), the resistance to bacterial degradation of the glucans was considerable and similar to that of pure cellulose. The β -glucans in refined rice and barley, with a higher amount of soluble fibre (50-60%), were more extensively degraded.

The purified cellulose added to some of the diets might have affected the degradation of fibre from the cereals. Van Soest et al.²¹ have shown that a previous intake of purified cellulose decreases the breakdown of cell-wall material in subsequent meals in humans. However, in this investigation two levels of pure cellulose in the diets, i.e. amounts corresponding to those added to the cereal diets, showed the same fermentability. This indicates that fermentation is independent of the amount ingested, at least at the concentrations applied in the present study. This is in agreement with Kies et al.²² who found a cellulose recovery of about 75% when diets containing different levels of cellulose were given to humans. Similarly, two levels of wheat bran, contributing 5 and 10% dietary fibre, were both 40% fermented²⁰. Furthermore, in the present study, dietary fibre in wheat flour, showed similar susceptibility to micro-organisms, whether purified cellulose was added or not. These data justify the correction

applied for purified cellulose in faeces (82% of ingested cellulose measured as glucose) when calculating the fermentability of cereal fibre glucans.

A considerable amount of the glucans found in faeces was degraded by amyloglucosidase and thus represents unabsorbed and unfermented starch. The faecal excretion of starch was most pronounced in the groups fed wheat flour, but starch was also observed in groups fed sorghum and whole-grain rye and barley flours. With refined wheat flour in diets, approximately 60% of the glucans excreted in faeces was starch. This is important when calculating the fermentability of non-starch polysaccharides. In relation to the starch intake, however, the faecal starch is almost negligible, representing less than 1% of the ingested starch.

In earlier studies with rats fed with different wheat flours, very small amounts, or no starch at all, were recovered in faeces^{4,18}. One might consider the possibility that the starch residues found in faeces originated from incompletely-digested potato starch. Fleming and Vose²³ found a low digestibility of raw potato starch in rats, but on the other hand, cooked potato starch was completely digested. In the present study no starch could be found in faeces when the diets contained only autoclaved potato starch or autoclaved potato starch and cellulose. This shows that all the potato starch was digested and absorbed and thus the starch found in faeces was in fact incompletely digested cereal starch.

The extent of intestinal digestion of starch in man is a matter of much discussion at present^{24,25}. The presence of amylose-lipid complexes in cereals has been reported by Acker and Becker²⁶. Amylose-lipid complexes reduce the solubility of amylose and render a fraction of the starch less available to α -amylase in vitro²⁷. On the other hand, these complexes are completely hydrolyzed by the thermostable α -amylase, Termamyl, at high temperature, in vitro²⁸. It has also been shown that amylose-lipid complexes between egg yolk lysolecithin and potato amylose are apparently completely digested and absorbed in the small intestine of rats²⁸. However, the difference between complete and nearly complete digestion and absorption is hard to determine with the experimental method used by those authors, and in the present investigation the faecal excretion of starch was very

small in proportion to the starch intake. Thus, the starch found in faeces might be the residue of incompletely digested amylose-lipid complexes. On the other hand, starch analyses in faeces with and without Termamyl gave very similar results, which indicates that the starch residue did not arise from amylose-lipid complexes.

Other authors^{25,29} have discussed the possibility of malabsorption of carbohydrates. Anderson et al.²⁹ proposed, by measuring breath hydrogen, that as much as 10-20% of the carbohydrates after wheat bread and pasta was malabsorbed in humans and suggested that complexes with gluten caused the malabsorption. Stephen et al.²⁵ concluded that on average 10% of the ingested starch, after a normal starch-containing diet, reached the colon in humans.

Lignin is usually reported to be resistant to bacterial degradation in the large intestine^{4,30}. This is in agreement with our results on whole-grain wheat, barley and sorghum flours. For rye and rice, however, faecal lignin values were higher in faeces than in feed. Southgate et al.³¹ reported the presence of lignin in faeces when no lignin had been consumed. They suggested that the lignin-like material found was of bacterial origin. It should be noted however that measurement of 'Klason lignin', is an unspecific procedure.

In conclusion, the present study has shown that dietary fibre from whole-grain cereals is more resistant to fermentation in the intestinal tract than that from refined flours. The larger proportion of soluble fibre and lower lignin content of the fibre seem to influence the availability for fermentation. The dietary fibre of whole-grain rice flour (including the husk) was extremely resistant, whereas that of other cereals showed a similar degree of fermentation (30-45%). Dietary fibre from refined flour of sorghum was relatively resistant to fermentation, only 22% being fermented, whereas refined flours of the other cereals fermented more extensively (65-90%).

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Table 1. Feed intake, fibre intake, faecal dry weights and dry-matter digestibilities for rats fed on diets containing cereal flours of different extraction rates.

Flour (extraction rate)	Feed intake (g/5d) Mean±SD	Cereal fibre intake (g/5d) Mean±SD	Faecal dry weight (g/5d) Mean±SD	Faecal dry weight ^a (g/5d)	Dry matter digestibility (%) Mean±SD
Basal diet	50 ± 0		0.8 ± 0.1	0.8	98.4 ± 0.3
Basal diet + 0.7 % cellulose	50 ± 0		1.2 ± 0.3	0.9	97.5 ± 0.5
Basal diet + 2.0 % cellulose	50 ± 0		1.9 ± 0.4	1.1	96.2 ± 0.8
Wheat (66) ^b	46 ± 2	0.8 ± 0.0	1.4 ± 0.1	1.4	96.9 ± 0.3
Wheat (100) ^b	50 ± 0	3.9 ± 0.0	5.4 ± 0.8	5.4	89.1 ± 1.5
Wheat (66)	50 ± 2	0.8 ± 0.0	2.7 ± 0.2	2.2	94.6 ± 0.4
Wheat (100)	50 ± 0	3.8 ± 0.0	6.0 ± 0.2	5.4	88.2 ± 0.4
Rye (65)	49 ± 1	3.4 ± 0.1	3.5 ± 0.1	3.5	92.9 ± 0.2
Rye (100)	50 ± 0	5.9 ± 0.0	7.2 ± 0.4	6.8	85.6 ± 0.8
Barley (69)	50 ± 0	3.8 ± 0.0	2.7 ± 0.6	2.7	94.5 ± 1.2
Barley (100)	50 ± 0	8.1 ± 0.0	9.4 ± 1.1	9.2	81.2 ± 2.3
Sorghum (63)	38 ± 3	0.6 ± 0.0	2.4 ± 0.3	1.8	93.6 ± 0.5
Sorghum (100)	47 ± 2	2.6 ± 0.1	4.9 ± 0.4	4.2	89.4 ± 1.0
Rice (64)	49 ± 2	0.5 ± 0.0	0.8 ± 0.2	0.8	98.3 ± 0.4
Rice (100)	50 ± 0	8.2 ± 0.0	10.4 ± 0.7	10.4	79.0 ± 1.4
Maize (fine)	47 ± 6	2.2 ± 0.3	2.5 ± 0.6	2.5	94.7 ± 0.8
Maize (whole)	48 ± 3	4.3 ± 0.3	5.2 ± 0.3	5.2	89.2 ± 0.5

^aMean faecal dry weights corrected for 82 % of the added cellulose (see text).

^bWheat without extra cellulose.

Table II. Dietary fibre composition and faecal recovery in rats given diets containing wheat flour of different extraction rates, with (A) and without (B) extra cellulose in the diet. (Pooled samples from five rats)

Component	Dietary fibre composition (% of dry matter)		Faecal excretion					
	mg/5d		% of intake					
	66	100	66		100		66	
	A ^a	B	A ^a	B	A ^a	B	A ^a	B
Rhamnose	-	-	-	10	-	31	-	-
Arabinose	0.6	2.3	19	16	390	390	9	10
Xylose	0.8	3.4	32	17	500	420	10	7
Mannose	0.1	0.1	43	8	41	39	-	-
Galactose	0.2	0.3	27	21	63	86	-	-
Glucose ^b	0.7	3.1	(180)	(97)	(910)	(1030)	(69)	(40)
Uronic acids	-	0.3	70	59	660	700	29	25
Klason lignin ^c	-	2.0	18	5	150	89	-	-
Total dietary fibre	2.4	11.5	210	140	2420	2390	24	17
(Soluble fraction,								
% of total fibre)	(54)	(13)						

*** p<0.001, ** p<0.01, * p<0.05.
Whole-grain flour compared with refined flour, with a variation coefficient of 30 %.

^aThe excreted glucans were corrected for 82 % of added cellulose.

^bValues within parenthesis are not corrected for excreted starch.

^cResidue insoluble in 72 % (12 M) sulphuric acid.

Table III. Dietary fibre composition and faecal recovery in rats given diets containing rye flour of different extraction rates. (Pooled samples from five rats)

Component	Dietary fibre composition (% of dry matter)		Faecal excretion		
			mg/5d		% of intake
	65	100	65	100	
Extraction rate:	65	100	65	100	100
Rhamnose	-	-	-	-	
Arabinose	1.7	3.1	240	560	30 45
Xylose	2.1	4.6	180	650	18 35*
Mannose	0.3	0.4	30	74	
Galactose	0.2	0.4	55	120	
Glucose ^a	2.1	3.5	(355)	(800)	(36) (57)
			330	650	33 46 ^{n.s.}
Uronic acids	0.4	0.5	34	130	
Klason lignin ^b	0.3	2.1	350	1080	129
Total dietary fibre	7.1	14.6	1220	3260	36 56*
(Soluble fraction,					
% of total fibre)	(51)	(24)			

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ n.s. = not significant.
Whole-grain flour compared with refined flour, with a variation coefficient of 30 %.

^a Values within parenthesis are not corrected for excreted starch.

^b Residue insoluble in 72 % (12 M) sulphuric acid.

Table IV. Dietary fibre composition and faecal recovery in rats given diets containing barley flour of two different extraction rates. (Pooled samples from five rats)

Component	Extraction rate:		Dietary fibre composition (% of dry matter)		mg/5d		Faecal excretion		% of intake	
	69	100	69	100	69	100	69	100	69	100
Rhamnose	-	-	-	-	-	-	-	-	-	-
Arabinose	0.9	2.1	71	600	17	65	17	65	***	***
Xylose	1.1	3.9	130	1230	25	72	25	72	***	***
Mannose	0.3	0.4	25	79						
Galactose	0.1	0.2	25	87						
Glucose ^a	5.5	7.7	(360)	(2040)	(14)	(61)	(14)	(61)	***	***
			340	1930	13	57	13	57	***	***
Uronic acids	-	0.8	30	190						
Klason lignin ^b	-	3.5	-	1520						
Total dietary fibre	7.9	18.6	620	5640	16	99	16	99	***	***
(Soluble fraction, % of total fibre)	(51)	(22)								

*** p<0.001, ** p<0.01, * p<0.05.
Whole-grain flour compared with refined flour, with a variation coefficient of 30 %.

^aValues within parenthesis are not corrected for excreted starch.

^bResidue insoluble in 72 % (12 M) sulphuric acid.

Table V. Dietary fibre composition and faecal recovery in rats given diets containing sorghum flour of two different extraction rates. (Pooled samples from five rats)

Component	Dietary fibre composition (% of dry matter)		Faecal excretion	
	63	100	mg/5d	% of intake
Extraction rate:	63	100	63	100
Rhamnose	-	-	-	-
Arabinose	0.1	1.2	10	12
Xylose	0.1	1.0	25	180
Mannose	0.2	0.2	14	180
Galactose	0.2	0.2	6	27
Glucose ^a	1.4	3.4	(295)	32
Uronic acids	0.3	0.7	18	(500)
Klason lignin ^b	0.4	2.5	130	370
Total dietary fibre	2.7	9.2	450	65
(Soluble fraction,				640
% of total fibre)	(15)	(5)	1510	78
				89
				57 ^{n.s.}
				(51)
				38 ^{**}
				82

*** p<0.001, ** p<0.01, * p<0.05 n.s. = not significant.

Whole-grain flour compared with refined flour, with a variation coefficient of 30 %.

^a Values within parenthesis are not corrected for excreted starch.

^b Residue insoluble in 72 % (12 M) sulphuric acid.

Table VI. Dietary fibre composition and faecal recovery in rats given diets containing rice flour of two different extraction rates. (Pooled samples from five rats)

Component	Dietary fibre composition (% of dry matter)			Faecal excretion	
	64	100	mg/5d	mg/5d	% of intake
Extraction rate:	64	100	64	100	100
Rhamnose	-	-	-	-	
Arabinose	0.05	0.8	-	280	
Xylose	0.05	3.2	12	1280	83
Mannose	0.05	0.1	11	64	
Galactose	0.05	0.3	-	100	
Glucose ^a	0.8	7.4	(32)	(3410)	(8)
			32	3400	8
Uronic acids	0.1	1.2	-	230	
Klason lignin ^b	-	3.9	-	2720	
Total dietary fibre	1.1	16.9	55	8080	11
(Soluble fraction,					
% of total fibre)	(57)	(4)			

*** p<0.001, ** p<0.01, * p<0.05.
Whole-grain flour compared with refined flour, with a variation coefficient of 30 %.

^aValues within parenthesis are not corrected for excreted starch.

^bResidue insoluble in 72 % (12 M) sulphuric acid.

Table VII. Dietary fibre composition and faecal recovery in rats given diets containing two types of maize flour, whole maize and maize sieved to a particle size of less than 340 μ (fine maize). (Pooled samples from five rats)

Extraction rate: Component	Dietary fibre composition (% of dry matter)		mg/5d		% of intake	
	Fine	Whole	Fine	Whole	Fine	Whole
Rhamnose	-	-	-	-		
Arabinose	1.1	1.9	110	480	22	54**
Xylose	1.0	2.4	160	810	36	72**
Mannose	0.1	0.1	13	23		
Galactose	0.2	0.4	29	130		
Glucose ^a	1.2	2.5	(210)	(720)	(39)	(62)
Uronic acids	0.5	0.6	200	710	37	61*
Klason lignin ^b	0.8	1.4	150	250	42	38
Total dietary fibre	4.9	9.3	710	2580	32	59*
(Soluble fraction, % of total fibre)	(6)	(3)				

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.
Whole maize flour compared with fine maize flour, with a variation coefficient of 30 %.

^a Values within parenthesis are not corrected for excreted starch.

^b Residue insoluble in 72 % (12 M) sulphuric acid.

Extrusion Cooking and Dietary Fiber: Effects on Dietary Fiber Content and on Degradation in the Rat Intestinal Tract

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ABSTRACT

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Dietary fiber content in raw and in extruded wheat products was measured gravimetrically after enzymatic solubilization of protein and starch. Dietary fiber content of whole grain wheat increased slightly after extrusion cooking, whereas the fiber content in wheat flour only increased after processing under extreme conditions. When the heat-stable α -amylase Termamyl, used in the dietary fiber assay, was excluded, the increase in dietary fiber was more pronounced. A redistribution of insoluble to soluble dietary fiber was observed in all extruded wheat flour samples. In raw wheat flour, 40% was soluble, whereas in extruded wheat flour, 50–75%

was soluble. The redistribution in whole grain flour was less pronounced. The degradation of dietary fiber monomers in the rat intestine was very similar with raw and extruded whole grain wheat flour. However, the dietary fiber in extruded wheat flour was more extensively degraded than in the corresponding raw material. Fecal glucan excretion did not increase when extruded materials were fed, which indicated that altered starch was completely absorbed or fermented. Rats fed wheat flour extruded under severe conditions developed diarrhea.

Extrusion cooking is being used increasingly in the processing of cereal-based foods.

Cereals, an important source of dietary fiber, constitute about 40% of the total dietary fiber intake in Sweden (Arnbjörnsson et al 1982). A number of physiological effects have been attributed to dietary fiber, among them increased fecal volume, reduced transit time, improved glucose tolerance, and lowered plasma lipids (Vahouny and Kritchevsky 1982). However, very few data are available on the effects of different food processes on dietary fiber.

Many studies on the relation of physiological effects with the content and chemical composition of fiber have been published (Jenkins et al 1978, Jenkins 1979). The physical state of fiber is also important. For instance, the bile salt-binding capacity decreases with decreasing particle size (Mongeau and Brassard 1982). Furthermore, the milling of cereals increases the availability of digestible carbohydrates both *in vitro* (Greenberg 1976) and *in vivo* (O'Dea et al 1980). On the other hand, extrusion-cooked wheat bran was no less beneficial to glucose excretion in alloxandabetic rats, compared with raw bran.¹

Thermal processing can make a fraction of the starch less available to enzymes and thus increase the dietary fiber value² (Varo et al 1983).

By definition, dietary fiber resists the digestive enzymes in the gastrointestinal tract. In the large intestine, however, fermentation occurs through bacterial enzymes (Nyman and Asp 1982). Different kinds of dietary fiber are fermented to various degrees. Solubility, chemical structure, and particle size are factors that can be expected to influence the susceptibility to bacterial degradation.

The importance of the physical state of the fiber in relation to bacterial fermentation was demonstrated by Brodribb and Groves (1978). The susceptibility to fermentation in the colon of human subjects was inversely proportional to the size of the bran particles. In addition, Berg et al (1972) found that the breakdown of cellulose by highly cellulolytic bacteria was proportional to its surface area. Heller et al (1980) also demonstrated the importance of particle size. The moisture content in feces was significantly higher in humans given a coarse bran diet than in humans given a fine bran diet. Moreover, the bulking effect of bran is less pronounced with cooked bran than with raw (Wyman et al 1976).

Thus, processes such as extrusion cooking that involve heating in combination with homogenization could be expected to affect dietary fiber, in terms of both physiological properties and fiber analyses. A variety of products, including pregelatinized flours,

breakfast cereals, crisp-breads and weaning foods, are produced by high-temperature short-time (HTST) extrusion cooking. In the extruder, the raw material is subjected to intense mechanical shear through the action of rotating screws. Cooking occurs at high temperature and pressure and at low water content. The mechanical treatment completely disorganizes the original structure of the raw material. The new structure obtained has been referred to as "uniform plasticized dough" (Kervinen et al 1981). Technical reviews on extrusion cooking have been published by Harper (1981) and by Linko et al (1981).

The purpose of the present investigation was to study the effect of extrusion cooking on dietary fiber in wheat products. The dietary fiber content in raw and in extruded products was measured gravimetrically after enzymatic solubilization of protein and starch. Because all the methods used for determining dietary fiber depend on complete digestion of starch, the efficiency of different enzyme systems for starch degradation was also evaluated. Furthermore, the effect of extrusion cooking on the susceptibility of dietary fiber to bacterial fermentation was studied through balance experiments in rats.

MATERIALS AND METHODS

Materials

Wheat starch (A-starch, Raisio Factories Ltd, Finland), wheat flour (extraction rate approximately 80%), and whole-grain wheat flour (Vaasa Mills Ltd, Finland) were the materials used. The products were processed in a Creusot-Loire BC-45 twin-screw extruder under conditions described in Table I. The feed rate was 200 g/min except in one case in which a higher feed rate was used. Feed moisture content was 15 or 20%. Screw speed varied between 100 and 200 rpm. Set temperature of barrel was 150°C in all experiments. Mass temperature was from 161 to 180°C and was measured with a thermocouple inserted just before the die. The extruded materials and the raw whole grain wheat flour were ground to pass a 0.5-mm sieve. The extruded products were shaped like expanded ribbons, and the grinding was performed to enable comparison with the corresponding raw materials. The most severely processed wheat flour (WF4), as judged from the degree of browning, is considered overcooked.

Methods

Determination of dietary fiber. The dietary fiber content was analyzed gravimetrically after enzymatic solubilization of protein and starch. Two different enzymatic systems for starch degradation were used. One system, based on the method by Hellendoorn et al (1975), used only physiological enzymes. After gelatinization for 15 min at 100°C, enzyme digestion was performed with pepsin (Merck No. 7190) at pH 1.5 for 1 hr and with pancreatin (Sigma No. P-1750) at pH 6.8 for 1 hr. The dietary fiber was precipitated with

¹C. Nygren, G. Hallmans, L. Jonsson, and N.-G. Asp. 1982. Effects of processed rye and wheat bran on the glucose metabolism. Poster presented at International Symposium on Human and Animal Nutrition, Palmerston North, New Zealand, Abstr. 176.

²C.-G. Johansson, M. Sijeström, and N.-G. Asp. 1984. Dietary fiber in bread and corresponding flours. Formation of resistant starch. Unpublished data.

four volumes of 95% ethanol for 1 hr. Filtration in G-2 crucibles with Celite (0.5 g) as a filtering aid was used to separate the dietary fiber. The other method was described by Asp et al (1983). In addition to incubation with physiological enzymes, as above, a thermostable bacterial α -amylase (Termamyl 120L, Novo A/S, Denmark) was added during gelatinization. Insoluble fiber was recovered by filtration (G-2 crucibles, Celite as filtering aid). Soluble fiber was precipitated with four volumes of 95% ethanol and recovered by another filtration.

TABLE I
Process Conditions* During Extrusion Cooking of Starch, Wheat Flour, and Whole Grain Wheat Flour

	Wheat Starch		Wheat Flour				Whole Grain Wheat Flour		
	WF1	WF2	WF3	WF4	WGF1	WGF2	WGF3		
Mass feed rate including moisture (g/min)	200	350	200	200	200	200	200	200	
Feed moisture content (%)	15	15	15	15	15	20	20	20	
Screw speed (rpm)	200	150	100	150	200	100	150	200	
Mass temperature (°C)	180	161	161	171	171	164	166	166	

*Creusot-Loire BC-45 twin-screw extruder. Set temperature of barrel, 150°C. Die: diameter, 5 mm; capillary length, 27 mm; clearance die/endplate, 2 mm. Screw: combination Die-RCCCC-Feed; R, Reverse flight screw element; C, Compression screw element; corotating intermeshing screws.

TABLE II
Composition of the Diets (% dry matter)

Component	Wheat Starch		Wheat Flour		Whole Grain Wheat Flour	
	Raw	Extruded ^a	Raw	Extruded ^b	Raw	Extruded ^c
Wheat product	69.3	69.3	74.2	76.3	81.6	81.2
Casein	9.9	9.9	...	...	...	...
Sucrose	10.0	10.0	15.0	12.9	7.6	8.0
Corn oil	5.0	5.0	5.0	5.0	5.0	5.0
Minerals ^d	4.8	4.8	4.8	4.8	4.8	4.8
Vitamins ^e	0.8	0.8	0.8	0.8	0.8	0.8
Choline chloride	0.2	0.2	0.2	0.2	0.2	0.2

^aProcess conditions described in Table I.

^bFlour WF4 with process conditions described in Table I.

^cFlour WGF3 with process conditions described in Table I.

^dContaining, in grams: CuSO₄·5 H₂O 8.6, ZnSO₄·7 H₂O 32.0, KH₂PO₄ 7780, NaH₂PO₄·2 H₂O 4024, CaCO₃ 7600, KI 1.6, MgSO₄·7 H₂O 2000, FeSO₄·7 H₂O 18, MnSO₄·H₂O 80, CoCl₂·0.46, NaCl 2382.

^eContaining, in grams, menadione 25.0, thiaminhydrochloride 10.0, riboflavin 10.0, pyridoxin hydrochloride 5.0, calcium pantothenate 25.0, nicotinic acid 25.0, folic acid 1.0, inositol 50.0, *p*-aminobenzoic acid 5.0, 0.2, vitamin B12 cyanocobalamin 0.015, vitamin A 0.86, vitamin D 0.025, vitamin E 100, wheat starch 3765.

The dietary fiber residues were corrected for remaining protein (assayed with the Kjeldahl procedure and calculated as N × 6.25) and ash. All analyses were done in triplicate.

Gas-liquid chromatographic characterization of dietary fiber constituents. After acid hydrolysis, gas-liquid chromatography (GLC) of the neutral sugars as their alditol acetates was performed as described by Theander and Aman (1979). The hydrolysis was performed in 12M H₂SO₄ for 2 hr. The acid was then diluted to 0.36M and the hydrolysis continued by reflux boiling for 6 hr. Dietary fiber constituents were expressed as their polymer weights (0.9 × monomer weight).

Starch determination. Approximately 100 mg of sample was suspended in 1 ml of distilled water and gelatinized in a boiling water bath for 15 min. One-half milliliter of 0.2M sodium-acetate buffer, pH 4.75, and 5 μ l of amyloglucosidase (Boehringer Mannheim GMBH, suspension of 10 mg/ml) were added. The suspension was incubated at 60°C for 30 min. After centrifugation of the suspension at 4,000 rpm, the released glucose was determined with glucose oxidase peroxidase reagent (5.6 g of GLOX-Novum [Kabi-Diagnostica, Stockholm, Sweden] in 100 ml of 0.5M TRIS buffer, pH 7.0). The glucose oxidase incubation was performed at 37°C for 1 hr, and the absorbance was measured at 435 nm. Starch content was expressed as glucose × 0.9.

In some determinations 5 μ l Termamyl were added in the gelatinization step.

Paper chromatography. Ten grams of flour (dry matter) or 2 g of feces (dry matter) were extracted overnight in 80% (v/v) ethanol. The extract was filtered, evaporated, and dissolved in 10 ml of distilled water. Paper chromatography was performed on Whatman no. 3 paper. The following solvent system was used: ethylacetate/acetic acid/water, 3:1:1(v/v). Papers were stained with a silver dip reagent (Trevelyan et al 1950). Xylose, glucose, fructose, and maltose (50 μ g) were used as standards.

Animal Experiments

Animals and diets. Male, Sprague-Dawley rats weighing approximately 75 g were divided into groups of five and placed individually in metabolic cages. Food intake was restricted to 10 g of dry weight per day. Water was provided ad lib. After a four-day adaption period, feed residues and feces were collected during a five-day balance period (Nyman and Asp 1982). Feces were collected dry, removed every day, and frozen at -20°C. Feces were then lyophilized, weighed, and milled to pass a 0.4-mm screen, and stored at -20°C until analysis.

All diets contained 5% corn oil, 10% sucrose, 4.8% minerals, and 1.0% vitamins, including choline chloride (Table II).

In the case of wheat flour and whole grain wheat flour, the flour was used as protein source, 1.5% N (d.b.). Sucrose was added to adjust dry matter content. In the groups fed wheat starch, the protein source was casein (ANRC reference protein, ICN Nutritional Biochemicals) 1.5% N (d.b.) (Table II).

Dietary-fiber characterization. Dietary-fiber composition in feed and in feces was analyzed by GLC, as described above. The dietary fiber in feed was isolated by using only physiological enzymes. Analysis of dietary fiber constituents in feces was performed directly on pooled samples from five rats. Glucose

TABLE III
Enzymatic Gravimetric Assay of Dietary Fiber in Raw and Extruded Flours: Comparison Between Different Enzyme Systems

Enzyme System	Dietary Fiber (% dry matter) ^a			
	Wheat Flour		Whole-Grain Wheat Flour	
	Raw	Extruded (WF1)	Raw	Extruded (WGF3)
Physiological enzymes only (pepsin, pancreatin)	6.8 ± 0.4	9.1 ± 0.6***	14.5 ± 0.3	20.5 ± 0.8***
Corrected for remaining starch (amyloglucosidase)	4.2 ± 0.4	5.2 ± 0.6*	12.4 ± 0.3	15.1 ± 0.8**
Corrected for remaining starch (Termamyl, amyloglucosidase)	4.0 ± 0.4	3.9 ± 0.6	12.2 ± 0.3	13.5 ± 0.8*

^aMean ± standard deviation. Significantly different from raw material (Student's *t*-test). * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

TABLE IV
Dietary Fiber Content in Raw and Extruded Wheat Flours

Material	Dietary Fiber (% dry matter) ^{a,b}					
	Total	Total ^c	Insoluble	Insoluble ^c	Soluble	Soluble ^c
Raw wheat flour	4.0 ± 0.2	4.0 ± 0.2	2.3 ± 0.1	2.3 ± 0.1	1.7 ± 0.1	1.7 ± 0.1
Extruded products ^d						
WF1	3.8 ± 0.1	3.7 ± 0.1	1.7 ± 0.1***	1.7 ± 0.1***	2.1 ± 0.1**	2.0 ± 0.1**
WF2	4.2 ± 0.5	3.9 ± 0.5	1.2 ± 0.2***	1.2 ± 0.2***	3.0 ± 0.4**	2.7 ± 0.4***
WF3	4.3 ± 0.1	3.6 ± 0.1*	0.9 ± 0.1***	0.9 ± 0.1***	3.4 ± 0.1***	2.7 ± 0.1***
WF4	4.9 ± 0.2***	3.9 ± 0.2	1.1 ± 0.3***	1.1 ± 0.3***	3.8 ± 0.1***	2.8 ± 0.1***

^a Dietary fiber content was measured according to Asp et al (1983).

^b Mean ± standard deviation. Significantly different from raw material (Student's *t*-test). * = *P* < 0.05, ** = *P* < 0.01, *** = *P* < 0.001.

^c Corrected for remaining starch in the fiber residue (amyloglucosidase).

^d Process conditions described in Table I.

TABLE V
Composition of Dietary Fiber^a in Raw and Extruded Wheat Flours

Relative composition (%)	Dietary Fiber Composition			
	Raw		Extruded (WF4) ^b	
	Insoluble	Soluble	Insoluble	Soluble
Arabinose	20	13	10	26
Xylose	26	16	9	34
Mannose	3	...	3	...
Galactose	...	3	...	6
Glucose ^c	16	3	6	6
Total	65	35	28	72
Sum of polysaccharides (g/100 g of dry matter)	2.0	1.1	0.9	2.3

^a Dietary fiber content was measured according to Asp et al (1983).

^b Process conditions described in Table I.

^c Corrected for remaining starch in the fiber residue (amyloglucosidase).

values were corrected for the small amounts of free glucose and starch found in feces (approximately 15% of total glucose excretion).

RESULTS

Starch Solubilization and Dietary Fiber Content

In Table III, different enzyme systems for solubilization of starch have been compared for dietary-fiber determination in raw and in extruded wheat samples WF1 and WGF3. When only physiological enzymes were used, the dietary fiber content in extruded products was higher. The increment relative raw material was 2.3% in extruded wheat flour and 6.0% in whole grain wheat flour. After correction for remaining starch with amyloglucosidase, the increase in fiber content due to extrusion was reduced to 1% in wheat flour and to 2.7% in whole grain wheat flour. With Termamyl present in the gelatinization step during starch analysis, corrected dietary fiber values in wheat flour were similar before and after extrusion, about 4%. In extruded whole grain wheat flour, the dietary fiber content (13.5%) was still slightly higher than in the raw material (12.2%).

Dietary Fiber Content After Preincubation with Termamyl

Wheat flour. The content of total, insoluble, and soluble dietary fiber analyzed according to Asp et al (1983) is shown in Table IV. In samples WF1, WF2, and WF3 the total dietary fiber content was not significantly different from that of the raw material. In the most severely processed sample (WF4), total dietary fiber increased from 4.0 to 4.9%. This could be accounted for as starch, analyzed by extensive amyloglucosidase digestion. This altered starch was found in the soluble dietary-fiber fraction.

Extrusion-cooking caused a redistribution of insoluble to soluble dietary fiber. Thus, in raw wheat flour 40% of total fiber was soluble versus 50–75% in the extruded products. Even in the product processed under mild conditions (WF1), the redistribution was statistically significant.

Gas-liquid chromatographic analysis of fiber constituents

confirmed the solubilization observed in the gravimetric assay. Results with the most severely processed product (WF4) and the corresponding raw material are shown in Table V. The different fiber constituents were solubilized to approximately the same extent.

Whole grain wheat flour. The content of total dietary fiber analyzed according to Asp et al (1983) was somewhat higher in all the extruded whole grain wheat flours than in the raw material (Table VI). Only minute amounts of remaining starch could be detected in the fiber residues. The increase of about 1% occurred in the soluble-fiber fraction. In two of the samples, there was also a slight increase in insoluble fiber. In raw whole grain wheat flour, 15% of the total fiber was soluble versus about 20% after processing.

Degradation of Dietary Fiber in the Rat Intestinal Tract

Wheat starch. Except for glucose, the excretion of typical fiber constituents in feces of rats given a fiber-free diet with wheat starch was very low, less than 10 mg in five days. The fecal excretion of polyglucose was 51 mg with raw wheat starch, and somewhat higher, 84 mg, with extruded wheat starch. However, calculated on ingested starch, both raw and extruded wheat starch were completely digested, 99.8 and 99.7%, respectively.

Wheat flour. The content of the dietary fiber constituents arabinose, xylose, mannose, and galactose, measured with GLC, was very similar in raw and in extruded wheat flour (Table VII). However, the content of glucans in the fiber fraction, ie, the sum of fiber glucose and starch resistant to enzymatic degradation in vitro was highly dependent on the enzyme system used for solubilization of starch, as shown in Table III. This was particularly evident with extruded materials. When only physiological enzymes were used, the glucan content in extruded wheat flour was 5.3%, compared with only 2.9% in the raw material. When corrected for remaining starch available to amyloglucosidase, the glucan content decreased, but the level in the extruded product was still higher than in the raw material. With Termamyl present in the starch assay, similar glucan values were obtained before and after extrusion.

Dietary fiber in wheat flour was very susceptible to bacterial degradation in the rat intestine (Table VII). When extruded wheat flour was fed to rats, the fecal excretion of dietary fiber constituents was even lower than after raw flour, indicating a higher fermentability due to extrusion. The fecal recovery of the main components, arabinose, xylose, and glucose averaged 22% in raw wheat flour versus 12% after extrusion.

The balance experiment was performed on wheat flour extruded under mild conditions (WF1). The most severely processed flour (WF4) caused diarrhea, making it impossible to collect feces. To study whether the diarrhea was caused by the formation of undigestible oligosaccharides, paper chromatography of ethanol extracts was performed with flour and with feces from rats given raw and extruded wheat flours (WF1 and WF4). In extruded flours, a spot was observed that could not be detected in raw flour. The intensity of the spot was much weaker with the flour processed under mild conditions (WF1). The *R_f* value was close to that of xylose. Furthermore, an increased content of fructose could be detected in extruded materials. In feces, very similar patterns were

observed with all materials.

Whole grain wheat flour. The dietary-fiber composition measured with GLC was similar in raw and extruded products, except for glucose (Table VIII). As with wheat flour, the content of glucans was dependent on the enzyme system used for solubilization of starch. When Termamyl was present, the glucan content was similar in raw and extruded materials.

The fecal excretion, in milligrams, of dietary-fiber constituents, including glucose, was approximately the same before and after processing. Calculated as percent of intake, the fecal excretion of arabinose was 45% and that of xylose was 33%. If intake was corrected for remaining starch with Termamyl, the excretion of glucans was also similar, 46 and 52%, respectively.

DISCUSSION

Depending on the enzyme system used for solubilization of starch, different dietary fiber values were obtained in both raw and extruded products. The variability in fiber content was most pronounced after extrusion cooking. This was directly related to the glucan content in the fiber residues.

Without Termamyl, the glucan content in extruded products was considerably higher than in the corresponding raw material. In contrast, with Termamyl, the increase in glucan content due to processing was less pronounced. This could be due to the presence of amylose-lipid complexes in extruded products. Formation of amylose-lipid complexes has been reported during extrusion-

TABLE VI
Dietary Fiber Content in Raw and Extruded Whole-Grain Wheat Flours

Material	Dietary Fiber (% dry matter) ^{a,b}					
	Total	Total ^c	Insoluble	Insoluble ^c	Soluble	Soluble ^c
Raw whole grain wheat flour	13.1 ± 0.5	13.0 ± 0.5	11.1 ± 0.1	11.1 ± 0.1	2.0 ± 0.4	1.9 ± 0.4
Extruded products ^d						
WGF1	14.4 ± 0.2*	14.1 ± 0.2*	11.6 ± 0.2**	11.6 ± 0.2**	2.8 ± 0.2*	2.5 ± 0.2
WGF2	14.3 ± 0.2*	14.1 ± 0.2*	11.4 ± 0.1*	11.4 ± 0.1*	2.9 ± 0.2*	2.7 ± 0.2*
WGF3	14.2 ± 0.1*	13.9 ± 0.1*	10.9 ± 0.2	10.9 ± 0.2	3.3 ± 0.1**	3.0 ± 0.1*

^a Dietary fiber content measured according to Asp et al (1983).

^b Mean ± standard deviation. Significantly different from raw material (Student's *t*-test). * = *P* < 0.05, ** = *P* < 0.01.

^c Corrected for remaining starch in the fiber residue (amylglucosidase).

^d Process conditions described in Table I.

TABLE VII
Composition and Fecal Recovery of Dietary Fiber in Raw and Extruded Wheat Flour^a

	Composition of Dietary Fiber ^b (% dry matter)		Intake (mg in five days)		Fecal Excretion (mg in five days) (% of intake)			
	Raw	Extruded ^c	Raw	Extruded ^c	Raw	Extruded ^c	Raw	Extruded ^c
Arabinose	0.9	0.8	310	250	57	29	18	12
Xylose	1.1	1.1	390	320	85	34	22	11
Mannose	0.1	0.2	48	45	15	8	31	18
Galactose	0.2	0.2	75	66	34	17	45	26
Glucose	2.9	5.3	990	1,590	78	52	7	3
	1.1 ^d	2.4 ^d	360 ^d	710 ^d	78	52	22	7
	0.9 ^e	1.2 ^e	310 ^e	360 ^e	78	52	25	14
Total	3.2 ^e	3.5 ^e	1,160 ^e	1,130 ^e	270	140	23	12

^a Pooled samples from five rats.

^b Dietary fiber fractions were isolated by using physiological enzymes.

^c Flour WFI with process conditions described in Table I.

^d Corrected for remaining starch in the fiber residue (amylglucosidase).

^e Corrected for remaining starch (Termamyl, amylglucosidase).

TABLE VIII
Composition and Fecal Recovery of Dietary Fiber in Raw and Extruded Whole-Grain Wheat Flour^a

	Composition of Dietary fiber ^b (% of dry matter)		Intake (mg in five days)		Fecal Excretion (mg in five days) (% of intake)			
	Raw	Extruded ^c	Raw	Extruded ^c	Raw	Extruded ^c	Raw	Extruded ^c
Arabinose	2.9	2.7	1,170	1,060	530	490	45	46
Xylose	4.5	4.2	1,810	1,660	600	540	33	33
Mannose	0.2	0.2	61	80	25	24	41	30
Galactose	0.3	0.4	130	140	135	67	104	48
Glucose	5.6	10.0	2,280	4,000	630	650	28	16
	3.6 ^d	6.4 ^d	1,460 ^d	2,540 ^d	630	650	43	26
	3.4 ^e	3.1 ^e	1,380 ^e	1,240 ^e	630	650	46	52
Total	11.3 ^e	10.6 ^e	4,550 ^e	4,180 ^e	1,920	1,770	42	42

^a Pooled samples from five rats.

^b Dietary fiber fractions were isolated by using physiological enzymes.

^c Flour WFG3 with process conditions described in Table I.

^d Corrected for remaining starch in the fiber residue (amylglucosidase).

^e Corrected for remaining starch in the fiber residue (Termamyl, amylglucosidase).

cooking. According to Mercier (1980), these complexes are resistant to α -amylase during *in vitro* incubation at 37°C. However, Holm et al. (1983) recently showed that amylose-lysolecithin complexes were completely digested and absorbed in the rat small intestine. Physiologically, these complexes should therefore be looked upon as starch rather than dietary fiber. It was further demonstrated that amylose-lipid complexes were efficiently hydrolyzed by Termamyl. The complexes were also available to pancreatic α -amylase, but high enzyme levels and a long incubation time were needed to obtain complete digestion. The high efficiency of the thermostable α -amylase Termamyl is probably due to dissociation of the amylose-lipid complexes at high temperature (Ghiassi et al. 1982), thus increasing the susceptibility for enzymatic attack. When using Termamyl in the analytical procedure for determination of dietary fiber (Asp et al. 1983, Theander and Åman 1979), artifacts due to insufficient digestion of starch *in vitro* are therefore minimized. This point is particularly important when analyzing processed products.

The rat balance experiments indicated that the starch digestion was very efficient *in vivo*. The glucan content in feces of rats given extruded products was similar to that obtained with the corresponding raw materials. However, the technique used in the balance experiments does not allow us to distinguish between digestion and absorption in the small intestine and bacterial fermentation. It is interesting that the fecal excretion of glucans with wheat flour was only slightly higher than that obtained with raw wheat starch.

In the present study, a solubilization of dietary fiber was observed during extrusion cooking of wheat flour. The solubilization appears to be dependent on process conditions. In the wheat product (WF1) processed at a high feed rate, i.e., at a shorter residence time, the solubilization was less pronounced. Varo et al. (1983) compared different methods for the determination of dietary fiber in processed materials. With some of these methods, soluble and insoluble dietary fiber was redistributed in extruded wheat flour and in whole grain wheat flour. However, when results from all methods were combined, the redistribution was not significant. Thus, the solubilization effect was method dependent.

The digestibility of both raw and extrusion-cooked wheat starch was almost 100%. Only minute amounts of starch could be detected in rat feces with amylglucosidase. Thus, extrusion-cooking of pure wheat starch did not affect its digestibility. The excretion of polyglucose on a fiber-free wheat starch diet was 51 mg in five days. This is in good agreement with the basal excretion obtained with maize starch by Nyman and Asp (1982).

The balance experiments indicated that the dietary fiber in raw wheat flour was fermented to a high degree. However, extrusion-cooking of wheat flour further increased the fermentability. No such effect was obtained with extruded whole grain wheat flour. Our data thus indicate that extrusion cooking primarily affects the fermentability of the smaller fiber fraction connected with the endosperm.

Food processes that lead to an increase in the surface area of the product could be expected to increase the availability of fiber to fermentation. In extruded wheat flour, the solubilization of fiber might be a factor in increasing the availability to microorganisms. In general, soluble fiber is more easily fermented than insoluble fiber (Cummings et al. 1979, Nyman and Asp 1982). The significant increase in soluble dietary fiber in extruded wheat flour products could be interpreted as the breaking of chemical bonds. Degradation of fiber has been reported with other processes that involve shear at low moisture content. Thus, according to Assarsson et al. (1959), glycosidic bonds in cellulose may be broken during dry ball milling.

With whole grain wheat flour, the content of dietary fiber constituents in feces was very similar before and after extrusion. This indicates that certain chemical structures resist bacterial degradation in the large intestine in spite of the prominent homogenization during extrusion. These results are in agreement with a recent study³ in which the particle size, *per se*, did not affect

the fermentation of purified wheat bran in the rat.

Paper chromatography on raw and on extruded wheat flour (WF1, WF4) indicated an increase in fructose content due to processing. This is in agreement with earlier results with extrusion-cooked wheat flour (Chiang and Johnson 1977). The diarrhea observed in rats given severely processed wheat flour cannot be explained at present. Maillard-reaction products formed during heat treatment by a reaction between proteins and reducing sugars have not been reported to cause diarrhea.

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**Bulk Laxatives - Their Dietary Fibre Composition,
Degradation and Faecal Bulking Capacity in the Rat**

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ABSTRACT

Intestinal dietary fibre degradation and faecal bulking capacity of various bulk laxatives were investigated using balance experiments on rats. Nitrogen, fat and mineral excretion in faeces was also studied. The dietary fibre content of the various bulk laxatives was quite different, and in g/kg dry matter: ACO fibre tablets (barley and citrus pulp) 451, Fiberform (wheat bran based) 817, Inolaxol (Sterkulia gum) 696, and Vi-Siblin (Ispaghula husk) 533. The increase in faecal dry matter/g dietary fibre was similar with ACO fibre tablets, Fiberform and Vi-Siblin. Inolaxol gave a significantly ($p < 0.001$) higher faecal dry-weight increment, mainly due to an increased mineral excretion. Of the dry-weight increment 59-82% constituted undegraded dietary fibre. Thus, 68-97% of the fibre passed through the gastrointestinal tract without being degraded. All the bulk laxatives caused a similar increase in the faecal N content, whereas the increase in faecal lipids was most pronounced with Vi-Siblin. The water-holding capacity of faeces was more pronounced with Inolaxol and Vi-Siblin than that with ACO fibre tablets and Fiberform.

INTRODUCTION

The addition of dietary fibre to the diet causes an increase in faecal wet as well as dry weight (1). Bulk laxatives contain dietary fibre and are widely used in western countries. They are recommended instead of irritating laxatives. The source of dietary fibre in bulk laxatives differs and various laxatives can, therefore, be expected to have different bulking effects.

The increment of faecal dry weight is more pronounced with dietary fibre which is resistant to degradation in the intestine than with easily fermented dietary fibre (2,3). On the other hand, gel-forming soluble types of dietary fibre have pronounced effects on bile-acid, cholesterol and glucose metabolism (for review, see Vahouny & Kritchevsky (4)). Such effects have also been documented for Ispaghula based bulk laxatives (5,6). However, gum karaya (*Sterkulia*) was found not to affect glucose or cholesterol metabolism in man (7).

The chemical structure of the dietary fibre influences the bacterial breakdown (3,8,9,10). Lignin and cellulose are known to be resistant to bacterial degradation, while pectin and, to some extent, hemicelluloses ferment more easily. Factors that can be expected to affect the susceptibility to intestinal degradation and thus faecal weights are solubility and particle size of the dietary fibre. Soluble dietary fibre, such as gums, in general, ferment to a greater degree than insoluble dietary fibre (2,3). Prynné & Southgate (11) found that highly soluble Ispaghula fibre was fermented to a great extent in humans, but the susceptibility of *Psyllium* fibre to the rumen microflora reported by Van Soest (12) was quite low, 60% was degraded. On the other hand, Brown, Pringuer & Anderson (13) found that *Sterkulia*, a gum with a very

high capacity for binding water but rather insoluble, was not degraded in rats.

The importance of particle size was demonstrated by Brodribb & Groves (14). Coarse bran caused a significantly higher wet weight of faeces than finely ground bran.

The increase in dry-matter content of faeces when dietary fibre is included in the diet is due to undegraded dietary fibre and to increased excretion of protein, fat or minerals (15, 16). This is partly or mainly due to an increase in bacterial mass (17). The proportion of bacteria and remaining dietary fibre varies with various dietary fibre sources.

This investigation was carried out in order to obtain detailed information on intestinal degradation and the faecal bulking capacity of some bulk laxatives on the Swedish market by using balance experiments on rats. The excretion of nitrogen, fat and minerals was also studied. We chose four bulk laxatives, ACO fibre tablets, Fiberform, Inolaxol and Vi-Siblin, differing concerning source, structure, molecular weight and solubility of the dietary fibre. In addition, faecal excretion of minerals with Fiberform, with a low phytic acid content, was compared with that of ordinary wheat bran, studied earlier with the same experimental model.

MATERIALS AND METHODS

Balance experiments

The four bulk laxatives - ACO fibre tablets, Fiberform, Inolaxol, and Vi-Siblin - were milled to a particle size of less than 0.4 mm.

All diets contained (in g/kg dry matter) protein in the form of casein, 100; sucrose, 100; fat, 50; vitamins including choline chloride, 10; minerals, 50; and dietary fibre of the various bulk laxatives, 100. Maize starch was used to adjust the dry-matter content (3). In addition, as a control, a basal diet was prepared as described above, but without any dietary fibre.

The rats were divided into groups of five. After a 4 d adaptation period to the diet a 5 d experimental period followed, when feed residues and faeces were collected. Food intake was restricted to 10 g dry weight per day and water was provided ad lib. Faeces were collected daily, frozen at -20°C , lyophilized, milled to a particle size of less than 0.4 mm, and then analysed. Details concerning diets and rat experiments can be found in Nyman & Asp (3).

Analytical methods

The total dietary fibre content in the feed was measured with the enzymatic method of Asp et al. (18). The dietary fibre composition in the dietary fibre residue of the feed and in faeces was measured using gas-liquid chromatography for the neutral sugars (19) and a decarboxylation method for the uronic acids (20, 21). The hydrolysis was performed in 72% H_2SO_4 at room temperature for 2 h. The sulphuric acid was then diluted with water to 0.36 M and reflux boiled for 6 h (21).

The insoluble residue was measured as Klason lignin. All dietary fibre components were expressed as polysaccharides, i.e. monomer weight $\times$ 0.9. In addition, corrections for hydrolysis losses and detector response were made by running the procedure with a known amount of monomeric sugars.

The N content in faeces was analysed using the Kjeldahl method. The crude protein content was calculated as $N \times 6.25$.

The amount of faecal lipids was determined using the method of Sobel (22) with a few modifications. 1 g lyophilized faeces was suspended in 6 ml distilled water. After the addition of 300 μ l concentrated HCl, fat was extracted in ethanol petroleum ether, 1:4, v:v.

The ash content was determined by combustion of the sample at 500°C for 24 h.

Water-holding capacity in faeces was measured using a method similar to that of McConnel, Eastwood & Mitchell (23). To 1 g lyophilized and milled faeces approximately 20 ml distilled water was added. After mixing and shaking the sample for 1 h at 37°C it was centrifuged at 14,000 g for 1 h at 10°C. The supernatant was discarded and the pellet weighed, dried and weighed again. The difference between the wet and dry weights was calculated and used to determine the water-holding capacity.

The amounts of the minerals, Na, K, Fe, Cu, Ca, Mg, and Zn in faeces were determined using atomic-absorption spectrophotometry after dissolving of the sample in H_2SO_4 . These measurements were also performed on material obtained from an earlier investigation with wheat bran using the same balance experiments on rats as in the present investigation (Nyman, Asp, Cummings & Wiggins, unpublished results).

The starch content in faeces was determined as glucose, using a glucose oxidase peroxidase reagent (Glox Novum, Kabi Diagnostica, Stockholm)

after incubation of the sample with amyloglucosidase (Boehringer Mannheim GmbH) for 30 min at 60°C at pH 4.75. The glucose oxidase incubation was performed at 37°C for 1 h and the absorbance was measured at 435 nm.

The amount of free glucose in faeces was measured with the glucose-oxidase reagent described above.

Dietary fibre constituents excreted in faeces were corrected for basal excretion, i.e. the excretion of typical fibre saccharides when the rats were fed a practically fibre-free starch diet. The basal excretion was very small and without practical importance (3). In addition, the glucose values in faeces have been corrected for free glucose and starch residues amounting to 40-60 mg/5 d.

All analyses were done at least in duplicate. Dry-weight and nitrogen determinations were performed on samples collected from individual rats. Since the amount of faeces from individual rats was limited and the variation coefficient (the quotient between the standard deviation and the mean value) in faecal dry weights was small in the individual groups (5% for all groups except the group fed Vi-Siblin which had a variation coefficient of 15%) the dietary fibre composition, fat, ash, starch, water-holding capacity and trace-element determinations in faeces were carried out on proportionally pooled samples from five rats.

When determinations were performed on samples collected from individual rats, Student's t-test was used in the statistical evaluation. In determinations made on pooled samples, a variation coefficient $\leq 30\%$ was assumed and then Student's t-test was applied. This variation coefficient is, concerning the faecal excretion of fibre components, considerably higher than that earlier obtained in experiments with resistant types of fibre on individual rats (i.e. 4% for wheat bran (3), 8% for maize bran and 4% for barley straw, M.Nyman and N.-G.Asp, unpublished results). The variation coefficient of faecal protein has never exceeded 22% (3). Thus, a supposed variation coefficient of 30% should in fact be more than liberal in the statistical evaluation of the pooled samples in this study.

RESULTS

Faecal bulking

Addition of the bulk laxatives caused highly significant increases in faecal dry weight (Table I). Of the dry-weight increment 59-82% could be accounted for as undegraded dietary fibre. The differences in excreted fibre between the laxatives were not significant. The remaining part of the dry-weight increment was protein, fat and ash. Inolaxol gave the most prominent increase and a significantly higher ($p < 0.001$) faecal dry weight than with the other bulk laxatives, due to a significant increase ($p < 0.001$) in the mineral excretion.

The faecal protein content was similar for all bulk laxatives investigated but significantly higher ($p < 0.001$) than with the basal diet. Faecal lipids constituted 8-18% of the faecal dry weight when bulk laxatives were included in the diet. The amount of faecal fat was significantly higher with Vi-Siblin ($p < 0.01$) than with the other laxatives.

Since faecal wet weight cannot be accurately determined with the rat experiments used, the water-holding capacity was measured in dried faeces. When Vi-Siblin and Inolaxol were included in the diet, the faeces held 4 and 6 times more water respectively, than when ACO fibre tablets and Fiberform were given.

Dietary fibre degradation in the rat intestinal tract

ACO fibre tablets. This bulk laxative consists of extracts from barley and citrus fruits. The dietary fibre content was 451 g/kg dry matter and of this the soluble fraction constituted about 30%. The main monomeric components were arabinose, xylose, glucose, uronic acids and lignin (Table II).

The arabinose and the xylose, proceeding mainly from barley husk, were

resistant to intestinal degradation and 64 and 76% respectively were excreted in the faeces. The glucans, mainly cellulose, were not degraded at all and 101% was recovered in faeces. The uronic acids were more susceptible to the microflora and 38% was excreted in the faeces.

Fiberform. Fiberform is an enriched wheat bran, in which starch, proteins and phytic acid have been degraded by enzymatic treatment. This bulk laxative contained 817 g/kg dietary fibre and of this about 5% was soluble. The main constituents of the dietary fibre were arabinose, xylose, glucose and lignin in proportions normally found in wheat bran (Table III).

The faecal recovery of the arabinose was 69%, that of xylose 46% and that of glucose 65%. Thus, Fiberform seemed to be somewhat more resistant to fermentation than ordinary wheat bran, and 71% of the total fibre was recovered in faeces compared with 58% for ordinary wheat bran (3).

Inolaxol. This highly gel forming but rather insoluble (24) type of bulk laxative from gum karaya (*Sterkulia*) contained 696 g/kg dietary fibre with rhamnose, galactose and uronic acids as the main components (Table IV).

The faecal recovery of the uronorhamnan was total. The galactose was also very resistant and only 13% was degraded.

Vi-Siblin. Vi-Siblin contains dietary fibre from Ispaghula husk (*Psyllium* fibre), 533 g/kg. The main components in this water-soluble dietary fibre (25) were arabinose and xylose (Table V). The faecal recovery of these main components was 75% for arabinose and 61% for xylose.

Excretion of trace elements

The faecal excretion of minerals was higher with Inolaxol in the diet than with the other bulk laxatives (Table VI). However, the Inolaxol preparation contained considerable amounts of minerals (18%). The increase in Na, K, and Mg was most pronounced, and the excretion exceeded the mineral intake originating from Inolaxol. As expected, the excretion of Na and K was more pronounced with the gel-forming types of bulk laxatives, Vi-Siblin and Inolaxol. The excretion of Fe, Cu, and Zn with Fiberform, with a low content of phytic acid, was similar to that with wheat bran, but the excretion of Mg was only half that with wheat bran ($p < 0.05$).

DISCUSSION

The various bulk laxatives gave similar faecal dry-weight increment when fed in equivalent amounts regarding dietary fibre. The water-binding capacity of faeces, measured in vitro, varied considerably with different bulk laxatives.

In ACO fibre tablets, Fiberform and Vi-Siblin, 95-99% of the dietary fibre obtained with the gravimetric method of Asp et al. (18) could be accounted for as polysaccharides and lignin, but with Inolaxol only 81% could be determined (Table II-V). However, according to Eastwood, Brydon & Anderson (7), gum karaya has an unusual resistance to acidic hydrolysis, which probably explains the difference (Table IV). An incomplete acid hydrolysis of the polysaccharide is probably also the case when analysing faeces. Thus, a calculation of percent recovery of the fibre components in faeces would be correct and comparable with the other bulk laxatives.

The various bulk laxatives showed a considerable resistance to intestinal degradation, 68-97% of the fibre being undegraded. The faecal recovery of the dietary fibre with ACO fibre tablets, Fiberform and Vi-Siblin was quite similar and about 70%. Inolaxol, however, seemed to be completely resistant to fermentation. This is a highly branched polysaccharide from *Sterkulia* gum, consisting of galacturono-rhamnan chains to which galactose and rhamnose are attached (26). Our results are in accordance with those reported by Brown, Pringuer & Anderson (13) for rats, and by Eastwood, Brydon & Anderson (7) for man. Furthermore, Salyers et al. (27) and Ochuba & Von Riesen (28) showed that gum karaya (*Sterkulia*) was not degraded by species of the genus Bacteroides and the family Enterobacteriaceae, bacteria normally found in the human colon.

Our data imply that Vi-Siblin (Ispaghula husk) is also resistant to bacterial fermentation. This is in agreement with Sosulki & Cadden (29) who found a similar intestinal degradation of Psyllium fibre in the rat. However, Prynne & Southgate (11) found that the fermentation of Ispaghula-based fibre was more pronounced, 47-82%, in man.

According to Van Soest et al. (30), dietary fibre is generally more fermented in man than in the rat. However, a comparison of intestinal dietary fibre breakdown in rat and man (Nyman, Asp, Cummings & Wiggins, unpublished results) showed good correlations with dietary fibre resistant to bacterial degradation as well as with easily fermented dietary fibre. For instance, guar gum was completely fermented when using the same balance experiments on rats as in the present investigation.

ACO fibre tablets contained arabinose and xylose originating from extracts of barley. These dietary fibre constituents showed a similar extent of fermentation as in whole grain barley flour (31). In that study, we found 65 and 72% of the arabinose and xylose remaining in faeces, respectively. In addition, the faecal recovery of the uronic acids in ACO fibre tablets, which originate from citrus pulp, was similar to that of pure pectin studied earlier (3).

Inolaxol gave the most pronounced increase in mineral excretion, mainly of Na, K and Mg. The increased Na and K excretion may, at least partly, be secondary due to the high water-binding capacity. Inolaxol has a high content of uronic acids, which are known to complex with calcium and other cations (32, 33). According to Cummings et al. (2), however, the faecal excretion of calcium was not increased in man after the consumption of pectin. It has been suggested that Ca is released and probably absorbed (34) when pectin is fermented in the colon (2, 3). Thus, the high resistance to bacterial degradation of the uronic acids in Inolaxol might explain the higher excretion of minerals in faeces. The formation of complexes between metals and polysaccharides can be measured using potentiometric methods. In such an experiment it was shown that Inolaxol bound 70 and 32%

of added Cu and Cd, respectively, but the binding of the same minerals to Sterkulia gum alone was only approximately 15% (Nair, Asp, Nyman, Frølich & Persson, unpublished results). These results indicate that the Sterkulia gum, per se, did not have any pronounced effects on the formation of complexes with Cu and Cd. Thus, the higher mineral excretion associated with Inolaxol may be more related to the kaolin used as a constituent in that preparation. However, the present study does not permit a conclusion on possible effects of any of the bulk laxatives on the mineral balance in man.

There is a controversy about the effect of phytate and fibre on mineral, especially zinc and iron, absorption (35). Fiberform is an enriched and dephytinized form of wheatbran. The present study indicates decreased magnesium excretion due to dephytinization ($p < 0.05$).

The amount of faecal lipids was higher ($p < 0.01$) with Vi-Siblin than with the other bulk laxatives. It is well documented that other highly soluble dietary fibres, such as guar gum and pectin, increase the faecal fat. Moreover, a lowering of cholesterol levels in serum has been attributed to Vi-Siblin (5, 6). The increased excretion of fat and also protein in faeces when certain dietary fibre is included in the diet can be due to decreased digestion (36, 37) or to increased microbial synthesis of protein and fat.

In conclusion, the faecal dry weight increment per gram dietary fibre was similar with ACO fibre tablets, Fiberform and Vi-Siblin. The dietary fibre content, however, differed in the various bulk laxatives and to obtain the same faecal dry weight increment, the intake of the various bulk laxatives has to be quite different. The faecal dry weight increment with Inolaxol was significantly higher than with the other bulk laxatives, mainly due to an increased mineral excretion. Inolaxol also seemed to be less susceptible to the intestinal microflora than the other bulk laxatives.

Compared with earlier investigations with ordinary wheat bran, using the same balance experiments on rats and with equivalent amounts of dietary fibre as in this investigation (Nyman, Asp, Cummings & Wiggings, unpublished results), faecal dry weights were approximately 20% higher with ACO fibre tablets, Fiberform and Vi-Siblin, and 60% higher with Inolaxol. However, the cost of bulk laxatives was up to 30 times higher per gram fibre than the cost of bran.

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Table I
Effect of various bulk laxatives on faecal output of rats
(mean values and standard deviations)

	Faecal dry weight (g/5 d)					
	Dietary fibre ^{a,b}		Protein		Fat ^a	
	Total Mean	SD	Mean	SD	Mean	Ash ^a
Basal diet	1.4	0.4	0.1	0.3	0.3	0.5
ACO fibre tablets	6.2	0.3***	1.2	0.1***	0.5**	1.0**
Fiberform	5.9	0.3***	0.9	0.1***	0.5**	0.7
Inolaxol	7.8	0.4***	0.8	0.1***	0.6***	1.9***
Vi-Siblin	6.2	1.0***	1.0	0.1***	1.1***	0.7

*** p<0.001 ** p<0.01 * p<0.05, compared with the basal diet.

^a Analysed on pooled samples from five rats. The supposed variation coefficient is 30%.

^b Including basal excretion of "dietary fibre", starch and glucose, together <0.2 g/5 d

Table II

Composition and faecal recovery of dietary fibre.

ACO fibre tablets

(Pooled samples from 5 rats)

	Composition of dietary fibre (g/kg dry matter)	Intake (mg/5 d)		Faecal excretion ^a mg/5 d % of intake	
		Mean	SD		
Rhamnose	7	81	0	4	5
Arabinose	52	580	0	370	64
Xylose	79	880	1	670	76
Mannose	12	130	0	44	34
Galactose	14	150	0	43	29
Glucose	88	970	1	980 ^b	101 ^b
Uronic acids	52	580	1	220	38
Klason lignin ^c	123	1360	2	1120	82
Sum	427	4730	7	3450	73
Dietary fibre ^d	451	5000	7		

^a Corrected for basal excretion (rhamnose 12 mg/5 d, arabinose, xylose, mannose and uronic acids 5 mg/5 d, galactose 27 mg/5 d, glucose 68 mg/5 d)

^b Corrected for free glucose and starch (7 and 38 mg/5 d, respectively)

^c Residue insoluble in sulphuric acid (12M).

^d Measured gravimetrically using the method of Asp et al. (1983)

Table III
Composition and faecal recovery of dietary fibre.
Fiberform
(Pooled samples from 5 rats)

	Composition of dietary fibre (g/kg dry matter)	Intake (mg/5 d)		mg/5 d	Faecal excretion ^a % of intake
		Mean	SD		
Rhamnose	-			6	
Arabinose	158	960	0	660	69
Xylose	251	1530	0	710	46
Mannose	7	43	0	47	109
Galactose	13	79	0	63	80
Glucose	198	1210	0	790 ^b	65 ^b
Uronic acids	26	160	0	160	100
Klason lignin ^c	157	960	0	1080	112
Sum	810	4940	1	3520	71
Dietary fibre d	817	4980	1		

- ^a Corrected for basal excretion (rhamnose 12 mg/5 d, arabinose, xylose, mannose and uronic acids 5 mg/5 d, galactose 27 mg/5 d, glucose 68 mg/5 d)
- ^b Corrected for free glucose and starch (4 and 30 mg/5 d, respectively)
- ^c Residue insoluble in sulphuric acid (12M).
- ^d Measured gravimetrically using the method of Asp et al. (1983)

Table IV
Composition and faecal recovery of dietary fibre
Inolaxol
(Pooled samples from 5 rats)

	Composition of dietary fibre (g/kg dry matter)	Intake (mg/5 d)		Faecal excretion ^a mg/5 d % of intake	
		Mean	SD		
Rhamnose	107	690	60	710	103
Arabinose	2	13	1	12	92
Xylose	-	-		15	
Mannose	-	-		-	
Galactose	127	820	60	710	87
Glucose	17	110	7	60 ^b	55 ^b
Uronic acids	281	1830	160	1860	102
Klason lignin ^c	30	200	17	180	90
Sum	564	3660	310	3550	97
Dietary fibre ^d	696	4520	380		

- ^a Corrected for basal excretion (rhamnose 12 mg/5 d, arabinose, xylose, mannose and uronic acids 5 mg/5 d, galactose 27 mg/5 d, glucose 68 mg/5 d)
^b Corrected for free glucose and starch (14 and 43 mg/5 d, respectively)
^c Residue insoluble in sulphuric acid (12M).
^d Measured gravimetrically using the method of Asp et al. (1983)

Table V

Composition and faecal recovery of dietary fibre
Vi-Siblin
(Pooled samples from 5 rats)

	Composition of dietary fibre (g/kg dry matter)	Intake (mg/5 d) Mean SD	Faecal excretion ^a mg/5 d % of intake
Rhamnose	11	90 9	33
Arabinose	112	960 93	75
Xylose	245	2100 203	61
Mannose	17	145 14	34
Galactose	22	185 18	65
Glucose	24	210 20	100 ^b
Uronic acids	30	260 25	62
Klason lignin ^c	54	460 44	93
Sum	515	4410 427	68
Dietary fibre ^d	533	4560 440	

^a Corrected for basal excretion (rhamnose 12 mg/5 d, arabinose, xylose, mannose and uronic acids 5 mg/5 d, galactose 27 mg/5 d, glucose 68 mg/5 d)

^b Corrected for free glucose and starch (28 and 33 mg/5 d, respectively)

^c Residue insoluble in sulphuric acid (12M).

^d Measured gravimetrically using the method of Asp et al. (1983)

Table VI
Total excretion of trace metals in faeces from rats
given various bulk laxatives
(Pooled samples from 5 rats)

Dietary fibre source	(mg/5 d) a						
	Na	K	Fe	Cu	Ca	Mg	Zn
ACO fibre tablets	23***	24***	12	1.0	330	20**	3
Fiberform	19***	20**	12	0.6*	310	20**	3
Inolaxol	410	100	15	1.0	430	44	3
Vi-Siblin	52***	39**	11	0.8	290	13**	3
Wheat bran b	18	24	11	0.7	260	39	3

a Inolaxol compared with the other bulk laxatives, *** $p < 0.001$ ** $p < 0.01$ * $p < 0.05$
The supposed variation coefficient is 30%.

b Studied in an earlier investigation (Nyman & Asp, unpublished results)

